FEASIBILITY OF TABLET IDENTIFICATION BY DIRECT INJECTION ELECTROSPRAY IONIZATION HIGH RESOLUTION TIME OF FLIGHT MASS SPECTROSCOPY

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ABSTRACT

The objective of this work was to study the feasibility of identifying unknown tablet samples by directly introducing the sample as solution into the electrospray ionization port of a high resolution time-of-flight mass spectrometer (HRTOF-MS) without prior separation by liquid chromatography. Thirty-four known drugs were analyzed by this method, among which 65% were identified, 26% were identified with some condition and 9% could not be identified from their mass spectrum. A procedure for identification of an unknown tablet was proposed from this study as a sample screening method for unknown tablet identification.

KEY WORDS: TABLET IDENTIFICATION / DIRECT INJECTION / HIGH RESOLUTION TIME OF FLIGHT / MASS SPECTROSCOPY

56 pages

ความเป็นไปได้ของการวิเคราะห์เม็ดยาด้วยเทคนิคอีเล็คโตรสเปรย์ไอออไนเซชั่นไฮเรโซลูชั่นไทม์ออฟไฟลทแมสสเปกโตรเมทรีโดยการฉีดสารละลายตัวอย่างโดยตรง

FEASIBILITY OF TABLET IDENTIFICATION BY DIRECT INJECTION ELECTROSPRAY IONIZATION HIGH RESOLUTION TIME OF FLIGHT MASS SPECTROSCOPY

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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์ ในการศึกษาความเป็นไปได้ของการตรวจวิเคราะห์เม็ดยาด้วยเทคนิคอีเล็คโตรสเปรย์ไอออไนเซชั่นไฮเรโซลูชั่นไทม์ออฟไฟลทแมสสเปกโตรเมทรี โดยการฉีดสารละลายตัวอย่างโดยตรงเข้าไปยังส่วนของไอออไนเซชั่นพอร์ต โดยไม่ผ่านกระบวนการแยกสารบริสุทธิ์ด้วยเทคนิคโครมาโทกราฟี จากการศึกษาตัวอย่างจำนวน 34 ตัวอย่าง มี 65% สามารถตรวจวิเคราะห์ได้ง่าย 26% สามารถตรวจวิเคราะห์ได้อย่างมีเงื่อนไข และ 9% ไม่สามารถตรวจวิเคราะห์ได้ นอกจากนี้ ยังได้มีการเสนอกระบวนการตรวจวิเคราะห์เม็ดยาตัวอย่างด้วยเทคนิคนี้ซึ่งเป็นเทคนิคที่ง่ายสำหรับการตรวจคัดกรองตัวอย่างสำหรับการตรวจวิเคราะห์เม็ดยา

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LIST OF ABBREVIATIONS

°C	Degree Celsius
API	Atmospheric pressure ionization
cm^{-1}	Per centimetre
ESI	Electrospray ionization
g	Gram
GC	Gas chromatography
HPLC	High performance liquid chromatography
HR-TOF	High resolution time-of-flight
kV	Kilovolt
LC	Liquid chromatography
m/z	Mass to charge
MeOH	Methanol
mL	Milliliter
MS	Mass spectrometry
no.	Number
ppm	Part per million
TLC	Thin layer chromatography
UV	Ultra violet
V	Volt
v/v	Volume by volume
µl/h	Microliter per hour

CHAPTER I
INTRODUCTION

Misuse of drugs can lead to fatality, and in many instances there are the use of illegal drugs. Therefore, drugs found at crime scenes are generally considered as important evidence.

Drugs come in many forms: liquid, tablet, capsule or powder, among which, tablet is one of the most common form of drugs.

When tablets are found at a crime scene, it is required to be identified for its relevance to the crime. Rapid identification can lead investigation to proceed in the right direction.

Nowadays, advances in chemical analytical techniques make identification of almost all drugs possible. In normal forensic laboratory, tablet samples are first analysed to determine the class of compounds by screening test procedures, such as color tests or immunoassays. Then they are identified by specific identification technique, such as spectrometry.

The most accurate technique for compound identification at present is mass spectrometry. Mass spectrometers are usually coupled with a chromatographic separation instrument, such as liquid or gas chromatograph.

However, many techniques have been developed so that a mass spectrometer can be used for identification without prior separation, such as Desorption Electrospray Ionization-MS (DESI-MS), Direct Analysis in Real Time-MS (DART-MS) and Desorption Atmospheric Pressure Chemical Ionization-MS (DAPCI-MS) [1]. With these instruments, solid samples are directly introduced into the mass analyser, and no prior chromatographic separation is needed. The analysis usually requires no or little sample preparation.

A Liquid Chromatography (LC) instrument attached to a high resolution mass analyser with an Electrospray Ionization (ESI) interface is an instrument used for sample identification and many forensic laboratories possess this instrument. When comparing the LC-ESI-MS to the non-separation mass spectrometer, the LC-ESI-MS

can reliably identify the drug but it takes a longer time per analysis and the cost for each analysis is also higher due to the separation process.

The use of LC-ESI-MS instrument without the liquid chromatography separation is another choice for rapid analysis of drug samples such as unknown tablets found at crime scene.

Analyzing forensic samples by ESI-MS instrumentation can save time and expense for each analysis, when compared with the analysis using LC separation (LC-ESI-MS).

CHAPTER II
OBJECTIVES

The objective of this research is to study the feasibility of identifying unknown tablet samples by directly introducing the sample as solution into the ionization port of an Electrospray Ionization Mass Spectrometer and measuring the mass spectrum.

CHAPTER III
BACKGROUND AND LITERATURE REVIEW

This chapter gives the theoretical and experimental background to the study.

3.1 Forensic Evidence

Forensic Science is a branch of science which applies any branch of science for answering questions of legal disputes. This definition might be applied to any legal prosecution, be it criminal or civil. But it is more generally used with criminal prosecution.

In any prosecution, it is necessary to answer the *three questions* that always arise, which are: *first*, has a crime been committed, *second*, who is responsible, and *third*, is there enough evidence to charge the person and support a prosecution. Forensic Science is expected to provide information thatcontributes to the clarification of those three questions. [2]

According to *Locard's Principle*, the important basic principle of forensic science is *'every contact leaves trace'*. When a person is involved in a crime, materials from the person are left at the crime scene, and, vice versa, materials from the scene of crime are trasferred to the person. The exchanged materials can be discovered both at the person and at the scene of crime and these exchanged materials can be used as evidence for answering the three question above. Those exchanged materials are called *forensic evidence.*

Wide range of materials can be involved in a crime, for example, biological materials, chemicals, drugs, fingerprints, footwear impressions, toolmarks, firearms, explosives, paper documents, electronic files, etc. Therefore, many scientific skills are required for working with forensic evidence; such as, evidence collecting

technique, photographic skills, analytical technique, chemistry skills, biological skills, toxicological skills, computer and information technology based skills, etc.

This study involves analytical tachnique for drugs, which is one of the important skills inforensic science tasks.

3.2 Solid Forms of Drugs

There are several forms of drugs as solid.

3.2.1 Tablets

Tablets are made by compressing active ingredients with inactive materials called excipients. These include diluents, adhesives, binders, lubricants, disintegrants and dyes.

Nowadays both legitimate illicit products are largely made by highly automated machines.

Compressed tablets are made by using multiple sets of dies and punches. But the difference between the legitimate and illicit tablets is that, the *legitimate tablets* are made in qualified clean-room condition, with strict quality-assurance procedures. So there is little variation in physical appearance, content of active ingredients and bioavailability. On the other hand, the *illicit tablets* are often made in poor condition; some damage dies and punches can leave visible characteristics imperfection on the tablets, which can provide valuable information for investigation of their origin.

Illicit tablets usually have distinctive logo, may be logo of famous commercial organizations (automobile manufacturers, mobile phone makers, fashion) or cartoon characters or simple texts and symbols.

Molded tablets are made by pressing active ingredients, diluent and binder (gum or adhesives) into a mould and then allow it to set; however, molded tablets are not common as pharmaceuticals nowadays.

Sometimes tablets may be coated with layers of sucrose, a thin film of resin or a soluble wax. There are many reasons for making tablets as *coated tablets*;

such as to make the medicine more palatable, to protect the active ingredients from exposure to environment, to delay the absorption, or to extend the duration of action.

3.2.2 Capsules

Capsules are popular, because they are easier to swallow compared to tablets. Most capsules consist of two parts, a cap and body, which are made from gelatin.

Filling of capsules may be done by hand, but usually they are filled by highly automated machines. The content may be powder, controlled-release granules, herbal materials, or even liquid. Capsules are also marked with printed label on either the cap or body or both.

There are many special kinds of capsules; for example, capsules made from cellulose and printed with natural dyes to satisfy vegetarian: wider diameter capsules used for double-blind clinical trials: very small capsules are designed for preclinical studies.

Liquid or semi-liquids filled capsules may be one –piece soft gelatin capsules or a two-piece hard geletin capsules sealed with shellac sealing band.

3.2.3 Transdermal devices

Some drugs with suitable solubility characteristics or those requiring extended dosing may be designed to be delivered through the intact skin. And the dosage form may be cream, spray, or patches that resemble adhesive wound dressings.

3.3 Identification of Drugs in Solid Form

Identification of tablets and capsules usually starts with visual examination of physical characteristics; shape, size, color, marks, etc. For cases with sufficient characteristic features of the samples and there is no legal involvement, identification of the unknown by their physical appearance alone is satisfied.

But, there are many situations, where the identification of the unknown by their physical appearance alone is not enough, which cases are when the unknown samples do not have enough characteristic features, and when the identification is for

forensic purposes. In these cases chemical analysis; such as Infrared Spectrometry, Ultraviolet Spectrometry, Thin Layer Chromatography, High performance Liquid Chromatography, Mass Spectrometry or melting point test, is needed.

3.3.1 Tablet Identification by Their Physical Appearance

Identification by the physical appearance of tablets and capsules, if possible, is fast and easy, especially when the questioned tablet or capsules are likely to be legitimate products. These can be rapidly done with help of databases for solid dosage form identification, which contains information about tablets and capsules available in each country. For example, *TICTAC*: a UK commercial database, *IDENTIDEX*: a commercial text-only database of largely American products, etc.

In order to examine an unknown sample by some database, examiner will only have to fill the physical appearances of the sample or answer some question, and the computer will show the possible identities of that unknown sample. However, identification by these databases needs the sample to have sufficient characteristic features in order to get the satisfied identity from the database.

3.3.2 Tablet Identification by Chemical Analysis

Tablets and capsules contain both active ingredients and excipients, and many times amount of the excipients maybe even higher than that of the active ingredients. So, to avoid the interference from these excipients, the separation of active ingredients should be carried out prior to sample analysis.

For tablets and capsules, simple extraction may be done with acidic aqueous solution, basic aqueous solution or methanol alone, followed by filtering the extract in the solution.

After obtaining the extracted active ingredients, UV-visible spectrometry is used for screening the extract. If the sample shows absorption bands in UV or visible region, then a confirmatory identification will be carried out. The confirmatory tests should base on other physical or chemical principle, which may be melting point test, IR spectrometry, TLC, HPLC or Mass Spectrometry

According to SOFT/AAFS Forensic Toxicology Laboratories Guidelines 2002, two techniques based on different physical or chemical principles are required to

confirm the identification for forensic purposes is recommended to be the confirmatory test.

3.4 Forensic Identification of Tablet

In standard forensic laboratories, an unknown sample submitted will first be examined visually; details about the container and its content must be carefully noted. If possible, identification of tablet by their physical appearance can also provide useful clue.

In order to chemically identify the active ingredients of the unknown tablet, the active ingredients present in the tablet must be separated from excipients that made up the tablet.

Thin Layer Chromatography (TLC) systems can be used for screening for the group of substance whether it contains basic, acidic or neutral drugs. Sometimes GC or HPLC system can even be used for screening substance group.

Absorption maxima in UV or visible region can also guide analysis, by comparing the absorption spectrum to UV-visible absorption data.

Infrared spectrophotometry gives spectrum that could be compared to spectrum of standard substances. Same substances must give same spectrum characteristics in the 'fingerprint region' (1600-400 cm^{-1}). This similarity can identify the active ingredients.

Mass spectrometry, the most effective identification technique of the day, is routinely used in almost every forensic laboratory. Mass spectrometers are largely used by combining with separation instrumentation; LC, GC, HPLC, CE, etc., as a confirmatory identification test for questioned substances.

3.5 Electrospray Ionization High Resolution Time-of-Flight Mass Spectrometer (ESI-HR-TOF-MS)

This mass spectrometer comprises of two important features, an electrospray ionization source and a time-of-flight mass analyser.

3.5.1 Electrospray Ionization (ESI) Source

ESI is an ionization technique in the group of Atmospheric Pressure Ionization (API), and it is suitable with samples in *liquid-phase*. This type of ion source ionizes the samples at atmospheric pressure and then the ion is transferred into the mass analyser.

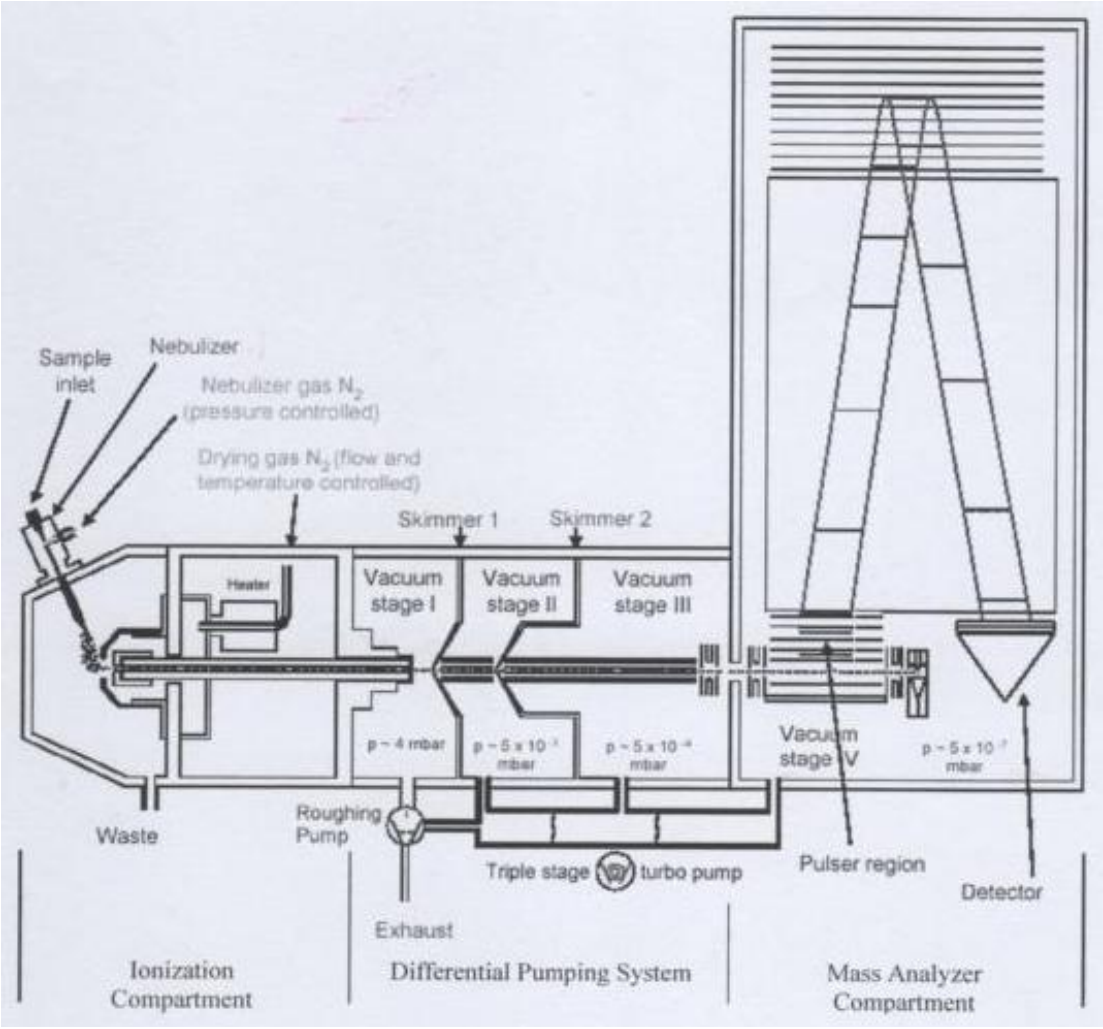

Figure 3.1 The schematic of an ESI-HR-TOF-MS

An ESI source ionizes the analyte sample in a liquid solvent. Sample solution enters ionization chamber through a metallic capillary. The schematic of an ESI-HR-TOF-MS is shown in figure 3.1. In the ionization chamber there is an electrostatic field obtained by applying a potential difference of 3-6 kV between the capillary tube and a counter-electrode. Both positive and negative ion mode can be

operated by switching the polarity of the potential. The liquid within the capillary is influenced by this electric field; ions in the solution are separated according to their charge, which cause charge accumulation at the tip of the capillary: positive charge ion mode and negative charge ion mode. As the electric force (Coulomb repulsion force) exceeds surface tension of the liquid at the tip of the capillary, the liquid will form a 'Taylor cone' and then small droplets are released from the liquid surface at the capillary tip [3]. The process of electrospray ionization is illustrated in figure 3.2.

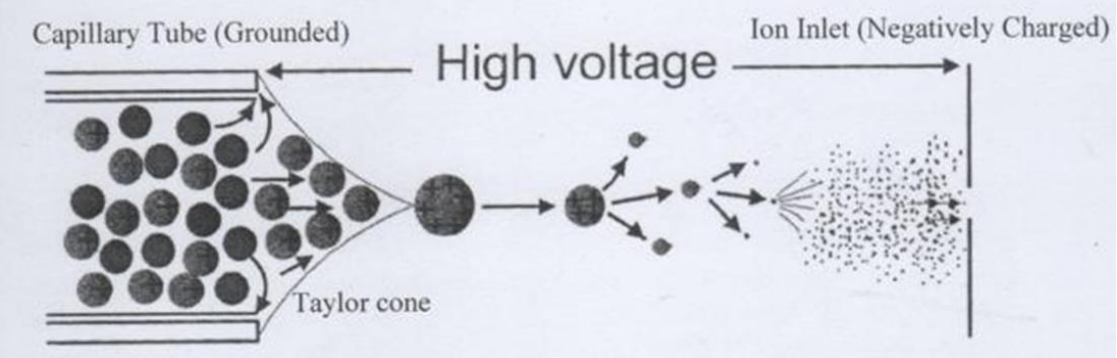

Figure 3.2 The process of Electrospray Ionization (Positive mode)

These small droplets travel further towards the counter electrode, which is at the ions inlet site. The droplets are exposed to heated drying gas that helps evaporating the solvent molecules in the droplet; which causes droplets to shrink, increasing charge per unit volume and the Coulomb repulsion force exceeds surface tension again, causing the droplets to explode and releasing many smaller droplets. These droplets explode further and further, until the final step that generates sample ions; by either desorption of ions from the droplet surface (for small molecules) or by evaporation of the last solvent molecules (for large molecules). Now the sample molecules that were in the liquid solution are changed to sample ions in gas phase at atmospheric pressure.

ESI has advantage that it can be easily coupled with separation instrumentation, such as; LC, HPLC, CE, etc. Furthermore, direct introduction of sample to mass spectrometer can also be done easily, because samples are introduced at atmospheric pressure interface.

However, the mass spectrometer that utilizes ESI must take into account coupling the two compartments with large pressure difference; which are the ion source compartment of atmospheric pressure and the mass analyser compartment of very low pressure (or high vacuum).

Coupling the two compartments is accomplished by using a differential pumping system; which comprises of a number of vacuum compartments that connected to a number of pumps, and placed between the ion source compartment and the mass analyser compartment, as illustrated in **Figure 3.1**.

After leaving the ionization compartment, the sample stream passes through these vacuum compartments, and then enters the analyser compartment. Sample enters from one compartment to another through an orifice: a small opening between the adjacent compartments, small enough to maintain the pressure difference and still sufficiently large to allow the ion transfer.

The highest efficiency of an ESI source is achieved by controlling both the pressure and the potential at each point along the ion path, on the instrument.

3.5.2 Time-of-Flight (TOF) Mass Analyzer

In TOF analyzer, ions are first accelerated by an electric field, and then they will be separated according to their velocities when they drift in a free-field region called a ‘flight tube’.

All ions acquire the same kinetic energy when accelerated by the electric field, but their velocities vary according to their mass; ions with higher mass having lower velocities and ions with lower mass having higher velocities. With this principle, once the time ions spend travelling a particular path is known, the mass of the ions can be calculated. Indeed, the mass-to-charge can be calculated.

Since TOF analyzer was invented, there has been much development to improve its efficiency; such as development of the delayed pulsed extraction that improve mass resolution, development of the Reflectron that also improve mass resolution, and development of the orthogonal accelerator that make TOF instrument able to combine also with the continuous ion sources beside combining with the pulsed ion sources.

3.6 Literature Review

Analytical techniques for forensic tasks in the present days are developing to be faster and more efficient to fulfil the increasing needs of society in accomplishing justice. Analysis of forensic samples without prior separation is another attempt to make analysis faster.

In a forensic identification of animal species that has largely been performed by biologists were successfully achieved by utilizing ESI-MS (Espinoza, 1998) [6]. In this study, analyses were done by using whole blood and dried blood without purification directly to obtain molecular weight of α- and β-protein present in each sample of haemoglobin. Among 980 samples 86% of the α/β pairs were useful for exclusion of all the other species.

Dietary supplements and herbal materials has long been an alternative option for health conscious people. While the consideration about the health risk associated with taking dietary supplements is another important topic. A screening method for the presence of synthetic phosphodiesterase type 5 inhibitors including sildenafil (as in Viagra®), tadalafil and vardenafil was developed by utilizing LC-ESI-MS technique (Gratz, 2004) [7]. The chemical quantification test of the samples was successfully accomplished.

Analysis of fire debris to detect the presence of arson accelerants is another important task of forensic scientists. Conventionally, these are done by sample extraction with activated charcoal strips and solid phase microextraction prior to analysis by GC-MS. But to reduce analysis time, a method utilizing Proton Transfer Reaction MS (PTR-MS) based on Time-of-Flight (TOF) detector was introduced (Whyte, 2007)[8]. Sample extraction and chemical pre-treatment steps were deleted, thus the analysis time was much less than the conventional GC-MS method: less than 5 minutes with PTR-TOF-MS comparing to less than 30 minutes for GC-MS.

DESI MS and DART MS is mass spectrometry technique, in which samples are directly ionized at the inlet of the MS instrument without sample pre-treatment or separation process. An example of its use in forensic laboratory is an analysis of genuine and fake artesunate antimalarial tablets. DESI and DART MS was used as the reference method in the study (Ricci, 2008)[9].

In coffee industry, quality of coffee beans affects quality of beverages. A technique to discriminate defective and non-defective coffee beans by using ESI-MS, was performed (Mendonca, 2008). Coffee beans were separated as defective and non-defective beans by their fingerprinting mass spectra that illustrate sucrose level of each type of coffee.

Writing ink analysis is usually required in many forensic cases. Several techniques are used for analyzing them; including both spectrometric and chromatographic methods. In 2009,Williams has developed an ESI-MS method for the analysis of dyes and vehicles in writing ink of ballpoint pens gel pens and rollerball pens. The technique allows efficient analysis of ink sample and requires minimal destructive sampling method [10].

Ayurvedic/herbal healthcare products (AHP) are considered safe because they are from natural resources. But there are reports that some of these practitioners unethically add synthetic drugs to AHPs. Analysis strategy for detection and verification of standard drugs, and AHPs was developed by using LC-MS/TOF (Savaliya, 2009) [11], and analysing in ESI mode was also employed. Efficient rapid screening method for steroid and anti-inflammatory drugs in AHPs was performed.

CHAPTER IV
MATERIALS AND METHODS

This chapter gives the important information about the materials used and how the study was preceded.

4.1 Instrumentation

4.1.1 Mass spectrometer

An ESI mass spectrometer Bruker micrOTOF PN236346 (Germany) was used. The instrument details are as follow;

Sample dlivery – By syringe pump with flow rate of 180 μl/h.

Ion source – Electrospray ionization source, operating at 200°C, end plate offset voltage was -500 V and capillary voltage was -4500 V.

Mode of analysis – Positive ion mode.

Analyzer – High resolution Tine-of-Flight analyzer

Scanning range – From 50 to 3000 *m/z*

4.1.2 Other instruments and materials

List of other instruments and materials are shown in Table 4.1.

Table 4.1 List of instruments and materials

Instrument/Material	**Model/Detail**
Micropipette	Eppendrof Model Research
Centrifuge	Vortex
Balance	One mg sensitivity
Pestle and mortar	Made of Porcelain
Filter paper	Whatman No.1

4.2 Chemicals and Samples

4.2.1 Chemicals

HPLC grade methanol and 1% aqueous acetic acid of various proportions was used as solvent. HPLC grade methanol was supplied from Burdick and Jackson (USA). Glacial acetic acid supplied from J.T. Beaker (USA) was dissolved to 1%(v/v) concentration in HPLC grade water.

4.2.2 Tablet samples

Tablet samples were collected from houses and offices since they represent tablets that can be found at crime scenes. Table 4.2 shows tablet samples tested in this study. Thirty-four tablet samples were tested; of which, 29 samples contained single active compound; the other 5 samples contained multiple active compound.

Table 4.2 Tablet samples tested in this study

Sample Name	**Active Ingredient(s)**	**Molecular formula**
Diazepam	Diazepam	$C_{16}H_{13}ClN_2O$
Hydroxyzine	Hydroxyzine	$C_{21}H_{27}ClN_2O_2$
Imipramine	Imipramine	$C_{19}H_{24}N_2$
Lorazepam	Lorazepam	$C_{15}H_{10}Cl_2N_2O_2$
Zolpidem	Zolpidem	$C_{19}H_{21}N_3O$
Aspirin A	Aspirin A	$C_9H_8O_4$
Aspirin B	Aspirin B	$C_9H_8O_4$
Bromhexine A	Bromhexine	$C_{14}H_{20}Br_2N_2$
Bromhexine B	Bromhexine	$C_{14}H_{20}Br_2N_2$
Ceterizine A	Ceterizine	$C_{21}H_{25}ClN_2O_3$
Ceterizine B	Ceterizine	$C_{21}H_{25}ClN_2O_3$
Chlorpheniramine A	Chlorpheniramine	$C_{16}H_{19}ClN_2$
Chlorpheniramine B	Chlorpheniramine	$C_{16}H_{19}ClN_2$
Mefenamic acid A	Mefenamic acid	$C_{15}H_{15}NO_2$
Mefenamic acid B	Mefenamic acid	$C_{15}H_{15}NO_2$

Table 4.2 Tablet samples tested in this study (cont.)

Sample Name	**Active Ingredient(s)**	**Molecular formula**
Omeprazole A	Omeprazole	$C_{17}H_{19}N_3O_3S$
Omeprazole B	Omeprazole	$C_{17}H_{19}N_3O_3S$
Roxithromycin A	Roxithromycin	$C_{41}H_{76}N_2O_{15}$
Roxithromycin B	Roxithromycin	$C_{41}H_{76}N_2O_{15}$
Amoxicillin A	Amoxicillin	$C_{16}H_{19}N_3O_5S$
Amoxicillin B	Amoxicillin	$C_{16}H_{19}N_3O_5S$
Amoxicillin C	Amoxicillin	$C_{16}H_{19}N_3O_5S$
Ibuprofen A	Ibuprofen	$C_{13}H_{18}O_2$
Ibuprofen B	Ibuprofen	$C_{13}H_{18}O_2$
Ibuprofen C	Ibuprofen	$C_{13}H_{18}O_2$
Paracetamol A	Paracetamol	$C_8H_9NO_2$
Paracetamol B	Paracetamol	$C_8H_9NO_2$
Paracetamol C	Paracetamol	$C_8H_9NO_2$
Paracetamol D	Paracetamol	$C_8H_9NO_2$
Cold tablet manufacturer A	Psuedoephedrine, Triprolidine	$C_{10}H_{15}NO$ $C_{19}H_{22}N_2$
Cold tablet manufacturer B	Paracetamol, Salicylamide, Clemizole, Phenylephrine	$C_8H_9NO_2$ $C_7H_7NO_2$ $C_{19}H_{20}ClN_3$ $C_8H_{13}NO_2$
Migraine headache tablet	Caffeine, Ergotamine	$C_8H_{10}N_4O_2$ $C_{33}H_{35}N_5O_5$
Muscle relaxant tablet	Paracetamol, Orphenadrine	$C_8H_9NO_2$ $C_{18}H_{23}NO$
Cold tablet manufacturer C	Paracetamol, Psuedoephidrine, Cholpheniramine	$C_8H_9NO_2$ $C_{10}H_{15}NO$ $C_{16}H_{19}ClN_2$

4.2.3 Tablet adulterants

A number of common tablet adulterants provided from the Faculty of Pharmacy, Mahidol University were tested. There are microcrystallide cellulose, magnesium stearate, polyvinylpirolidone, sodium starch glycolate, lactose and corn starch.

4.3 Preparation of Sample Solution

Both tablet samples and adulterants samples were prepared as dilute solution. Tablets were finely (for capsule samples, only powder content was used), while adulterants samples were in powder form. Each sample was weighed 0.010-0.020 g and dissolved in 10-20 ml of MeOH (HPLC grade) to give concentration of 1,000 ppm. This sample solution was then filtered through filter paper (Whatman no.1) to remove any remaining solid particles. This solution was then diluted to 100 ppm in 80:20 (v/v), methanol:1%aqueous acetic acid.

4.4 Data Analysis

Formulation of each standard tablet was known, and the active compound's monoisotopic (exact) mass calculated. From the exact mass of the active compound plus the mass of the hydrogen atom that will form adduct with the analyte molecule, it is possible to identify the *m/z* value in the observed mass spectrum.

A list of target ionized species' *m/z* value for each active ingredient is made, which is called 'Compound Target List'. By using the Compound Target List, a procedure for tablet identification was made.

CHAPTER V
RESULTS AND DISCUSSION

Results obtained from the study are shown and discussed in this chapter.

5.1 Mass Spectrum of the Solvent

Figure 5.1 shows the mass spectrum obtained when the solvent (MeOH:1%acetic acid 80:20 (v/v)) was introduced in to the mass spectrometer. Mass spectrum showed many peaks that are due to clusters of solvent molecules.

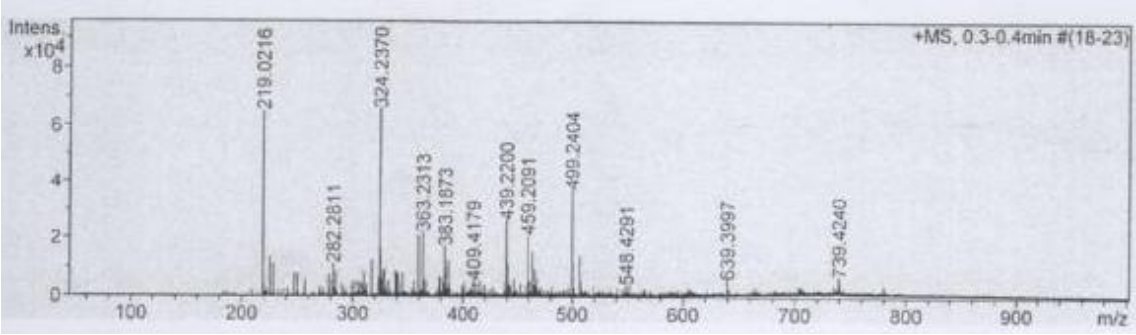

Figure 5.1 Mass spectrum of the solvent (MeOH:1%acetic acid 80:20 (v/v))

5.2 Mass spectrum of Some of the Adulterants

Figure 5.2 shows mass the spectra of binders commonly used in tablet formulates. There are microcrystallide cellulose, magnesium stearate, polyvinylpyrrolidone, sodium starch glycolate, lactose and corn starch. They are dissolved in 80:20 (v/v), MeOH:1% acetic acid, at 100 ppm powder concentration. The spectra showed many unidentifiable peaks.

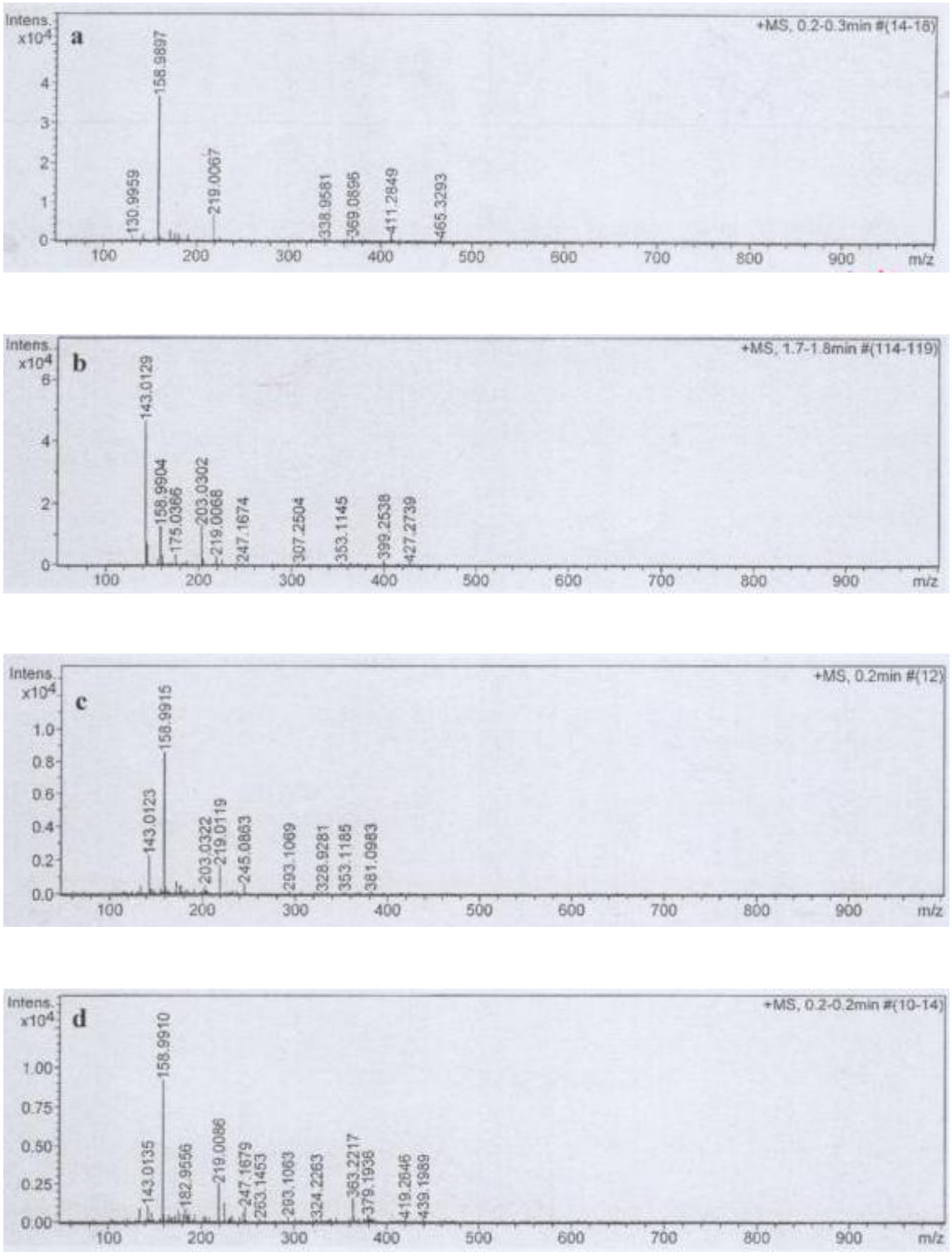

Figure 5.2 Mass spectra of six binders **a**) Microcrystallide cellulose, **b**) Magnesium stearate, **c**) Polyvinylpyrrolidone, **d**) Sodium starch glycolate, **e**) Lactose and **f**) Corn starch

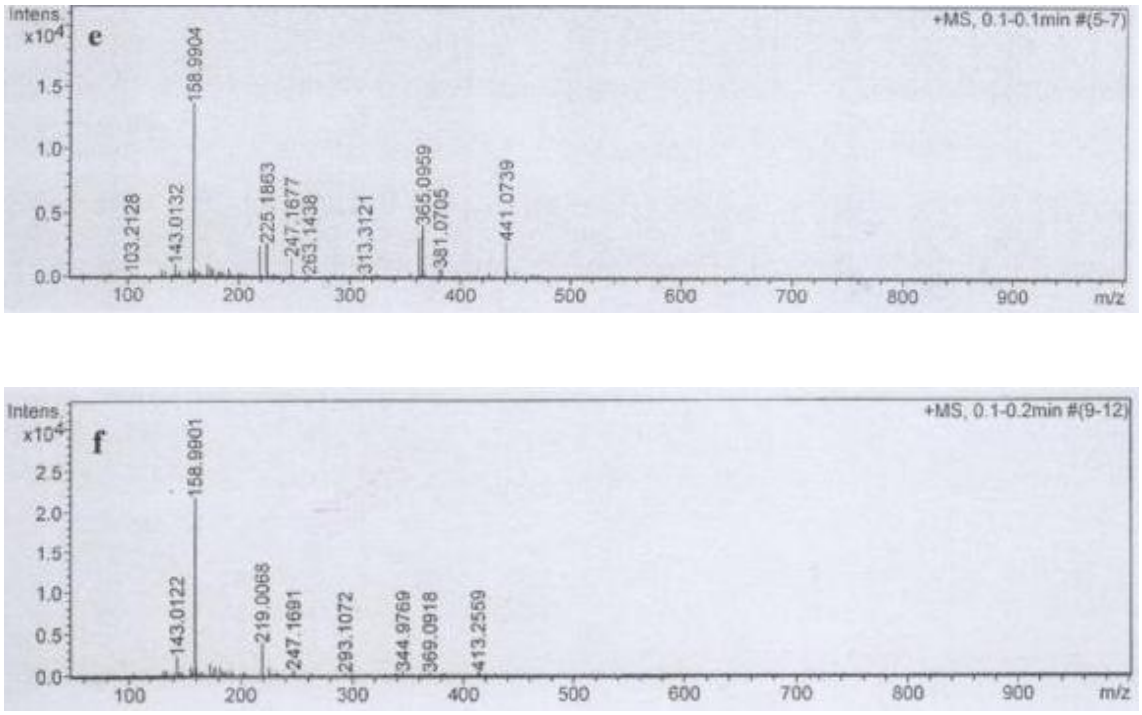

Figure 5.2 Mass spectra of six binders **a**) Microcrystallide cellulose, **b**) Magnesium stearate, **c**) Polyvinylpyrrolidone, **d**) Sodium starch glycolate, **e**) Lactose and **f**) Corn starch (cont.)

5.3 Optimum Composition of Sample Solution

5.3.1 Solvent composition

Three solvent compositions were tested using diazepam and chlorpheniramine tablets at 100 ppm; **1**)100% MeOH, **2**) 80:20 (v/v), MeOH:1% acetic acid, and **3**) 50:50 (v/v), MeOH:1% acetic acid. The mass spectra are shown in Figure 5.3.

Figure 5.3 **a1**, **a2** and **a3** showed *m/z* peak of protonated diazepam, diazepam+H^+ at 285.0794, 285.0791 and 285.0802, respectively. However, its spectrum had different intensity. In figure 5.3 **a1**, intensity was very small, compared to that of the base peak (*m/z* 365.1046). In figure 5.3 **a2** and **a3**, diazepam's peak was the base peak of the spectrum. Thus, the formulate of the tablet has a large affect on there mass spectrum; this is called the ion suppresser effect.

The spectra in figure 5.3 **b1**, **b2** and **b3**, showed *m/z* characteristic peak of protonated chlorpheniramine, chlorpheniramine+H^+ at 275.1309, 275.1317 and 275.1301, respectively. The intensities of the peaks in the spectra were again different. However, in all three spectra, the base peak was that of chlorpheniramine+H^+ peak.

The solvent composition of 80:20 (v/v), MeOH:1% acetic acid produced the base peaks of the protonated drug and was thus selected as the solvent for all further studies.

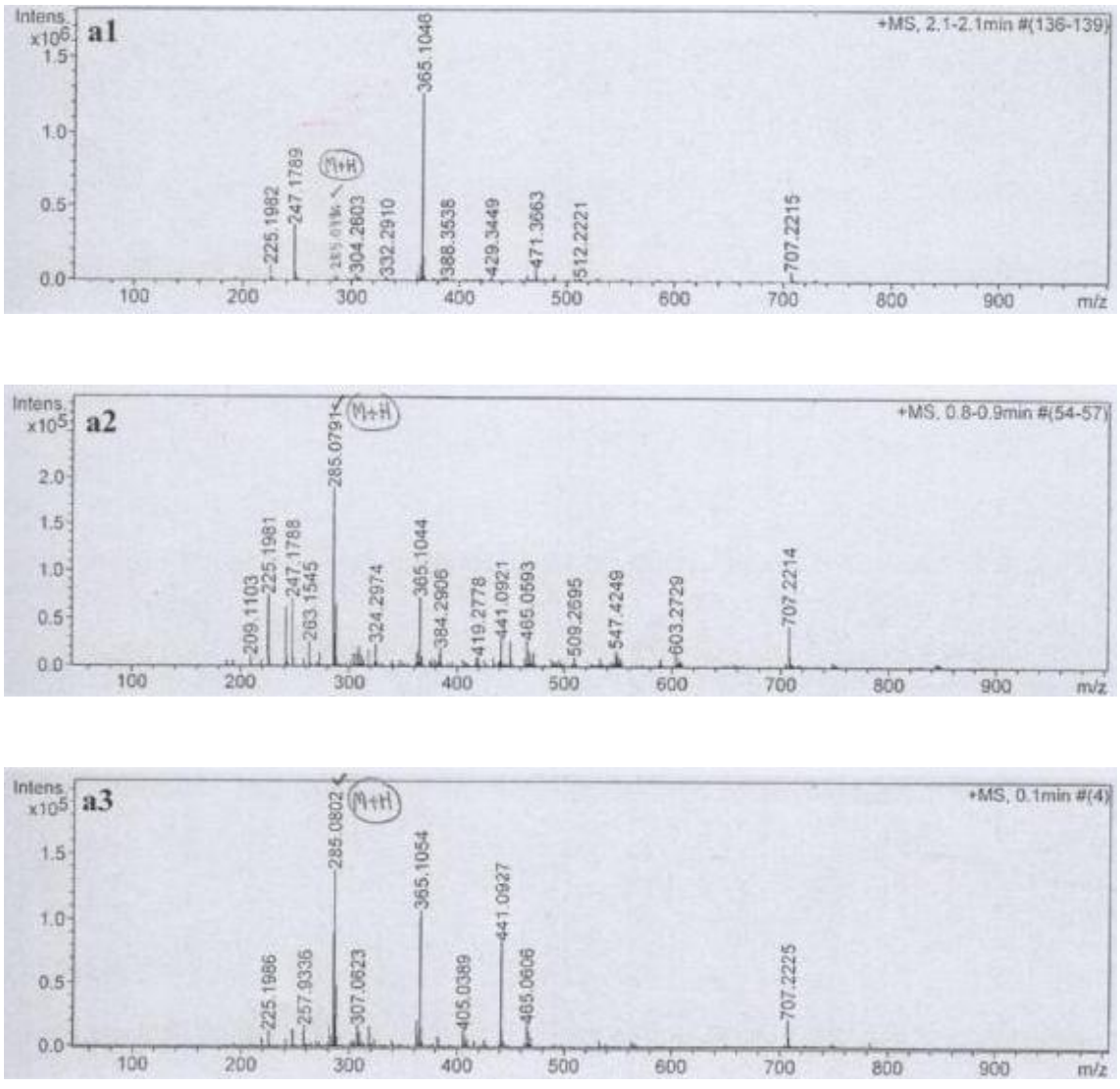

Figure 5.3 Mass spectra of **a1-a3**) Diazepam and **b1-b3**) Chlorpheniramine in three solvent compositions: 100:0, 80:20 and 50:50 % of MeOH:1% acetic acid, respectively. Tablet powder concentration is 100 ppm.

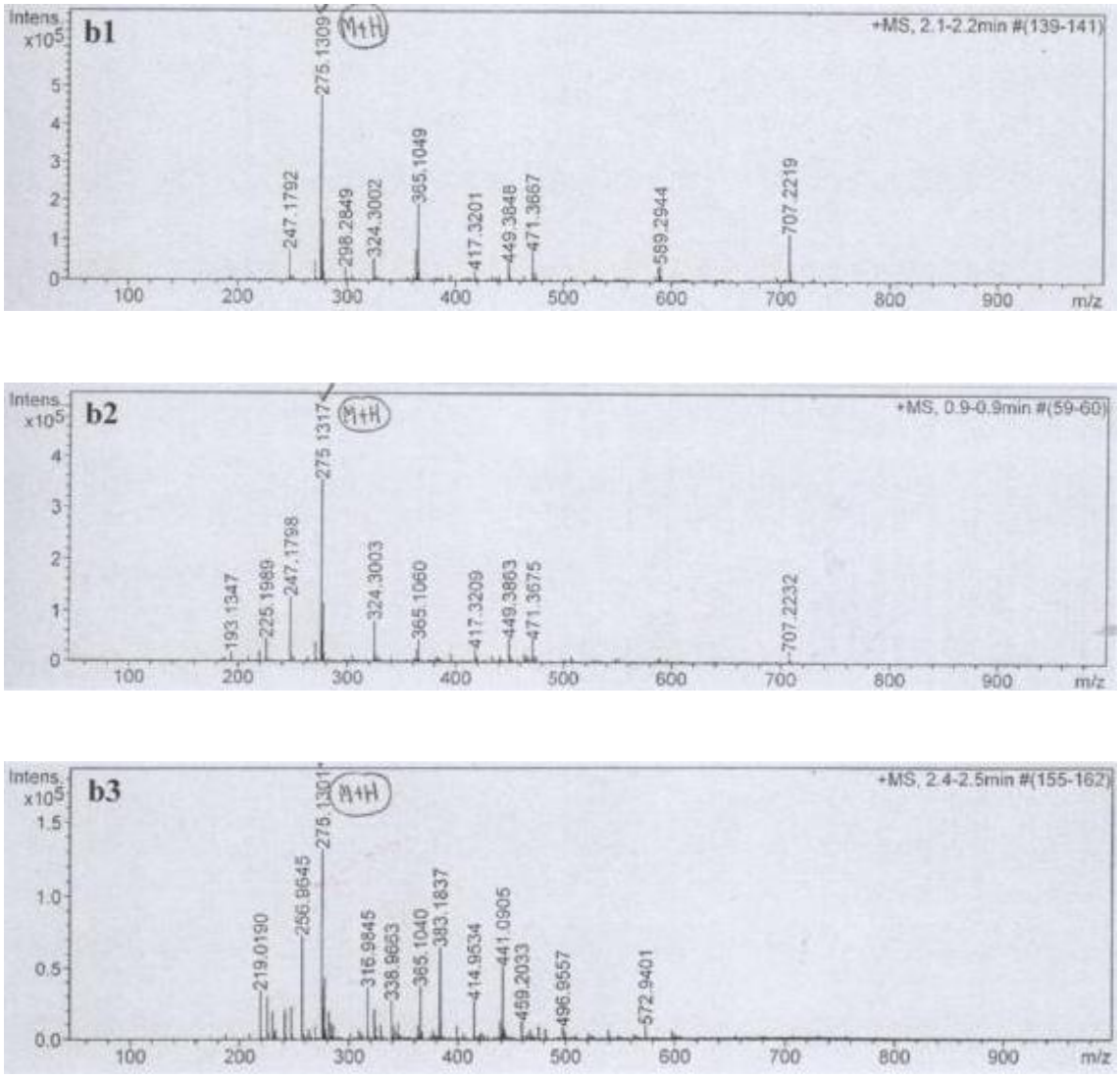

Figure 5.3 Mass spectra of **a1-a3**) Diazepam and **b1-b3**) Chlorpheniramine in three solvent compositions: 100:0, 80:20 and 50:50 % of MeOH:1% acetic acid, respectively. Tablet powder concentration is 100 ppm. (cont.)

5.3.2 Tablet powder concentration

Diazepam and paracetamol tablets were used for optimization of the concentration. Sample were prepared at 1, 50 and 100 ppm as solid powder dissolved in the optimized solvent composition (80:20 (v/v), MeOH:1% acetic acid). The mass spectra are shown in figure 5.4.

As expected, the peaks for the protonated compound increased with increasing concentration. This is observed in figure 5.4 **a1-a3**, for *m/z* 285.08 of diazepam+H^+.

For paracetamol, the sample at 100 ppm showed peaks for adduct of paracetamol with sodium ion, $[M+Na]^+$, *m/z*=174.06 and the dimer, $[2M+Na]^+$, *m/z*=325.12.

Thus all samples were prepared at concentration of 100 ppm.

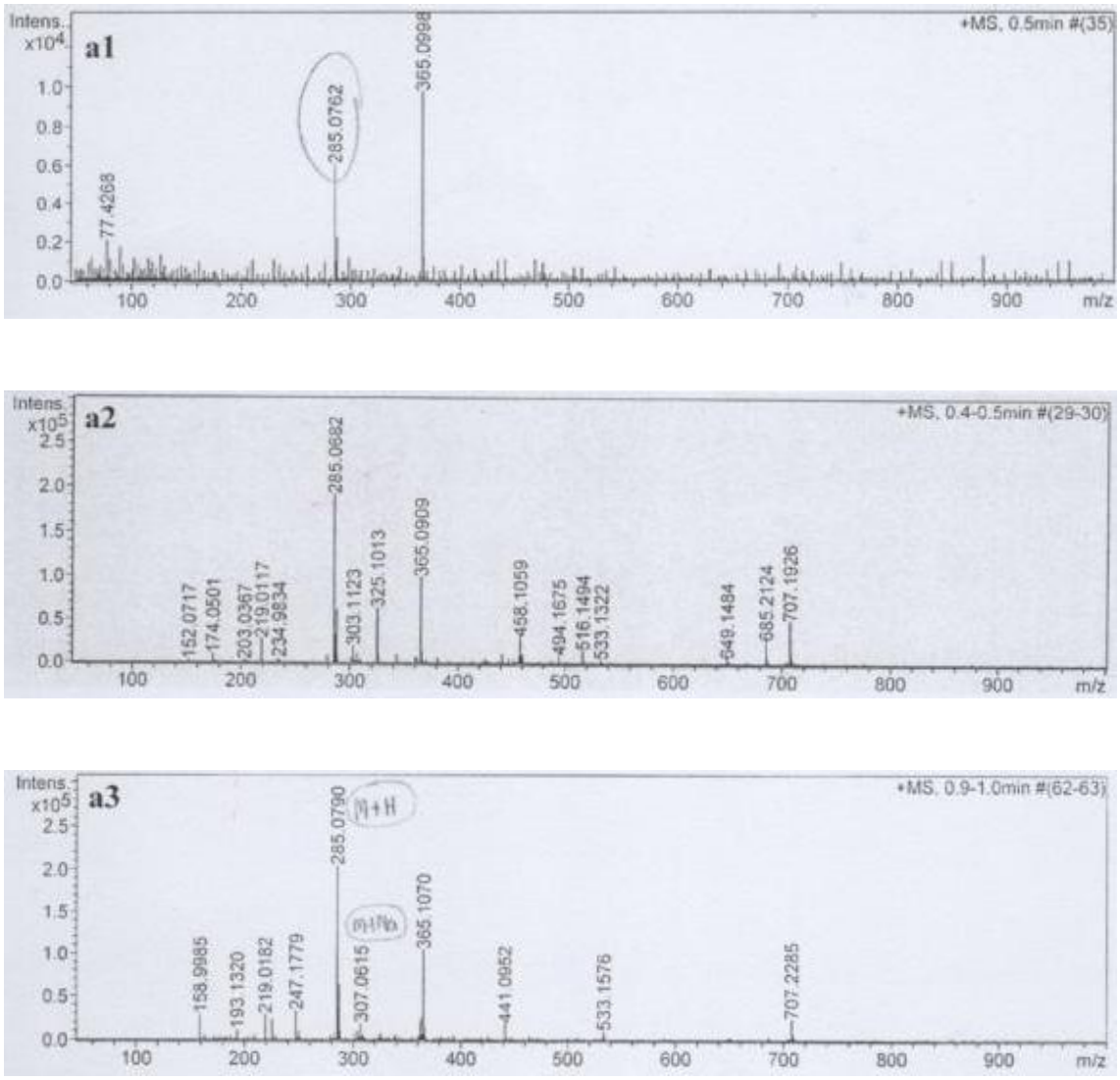

Figure 5.4 Mass spectra of **a1-a3**) Diazepam and **b1-b3**) Paracetamol of 1, 50 and 100 ppm tablet powder concentration, respectively. Solvent composition is 80:20 (v/v), MeOH:1% acetic acid.

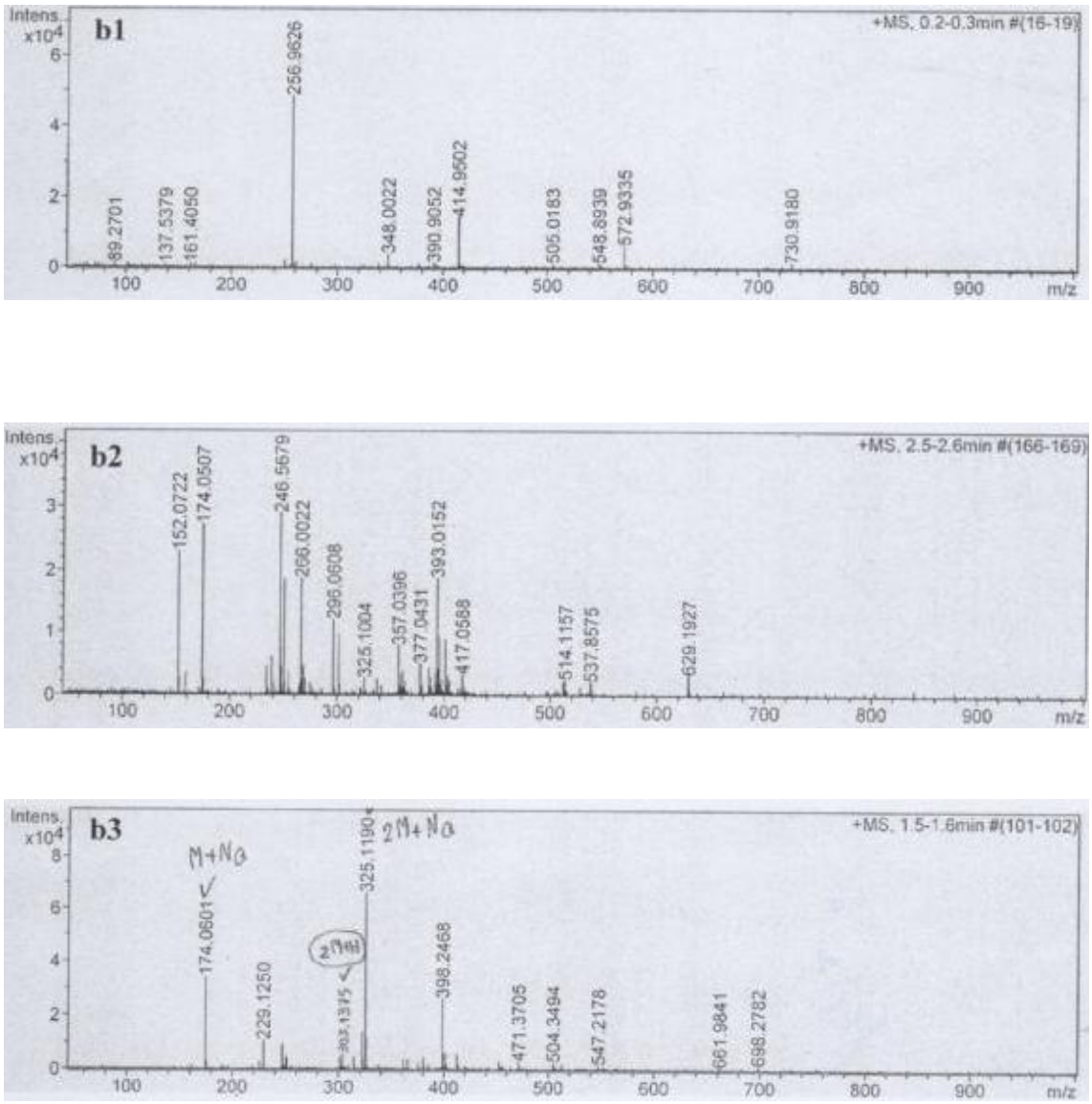

Figure 5.4 Mass spectra of **a1-a3**) Diazepam and **b1-b3**) Paracetamol of 1, 50 and 100 ppm tablet powder concentration, respectively. Solvent composition is 80:20 (v/v), MeOH:1% acetic acid. (cont.)

5.4 Analysis of the ESI Mass Spectrum

In this section, the mass spectra is of all the 34 tablet samples are shown and the data evaluated.

An ESI mass spectrum is a plot between signal intensity (molecular abundance) of molecular ionized species and its *m/z* value. In the ESI source, neutral molecules are not fragmented, but they form adducts by gaining H^+ or Na^+ (in positive

ion mode) or form dimers before gaining H^+ or Na^+. The charged species are not fragmented (soft ionization mode).

5.4.1 Diazepam ($C_{16}H_{13}ClN_2O$)

Employing the exact atomic mass of the most abundant isotope, the monoisotopic mass of diazepam molecule was calculated (M=284.0716 amu.). Hydrogen and sodium cations that are most frequently found to form adducts in ESI positive mode, have 1.007825 and 22.989770 amu., respectively.

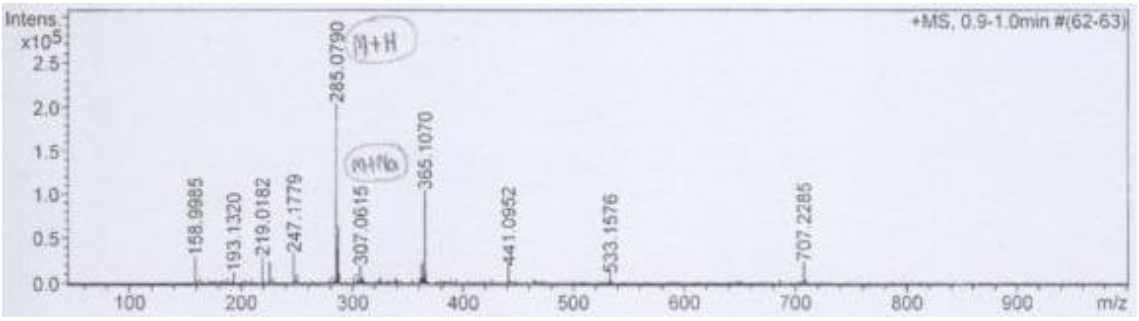

Figure 5.5 ESI mass spectrum of Diazepam tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

Consequently, the expected peaks in the diazepam spectrum can be identified. The possible target peaks of diazepam are $M+H^+$ at 285.08, $M+Na^+$ at 307.06, $2M+H^+$ at 569.15 and $2M+Na^+$ at 591.13 (M=monoisotopic mass of diazepam). The isotope distribution for one chlorine atom should be noted, i.e. $M+H^+$: $M+2+H^+$ and $M+Na^+$: $M+2+Na^+$ = 100 : 35.

From the diazepam spectrum (**Figure 5.5**), the peak at 285.0790 represents $M+H^+$ amd the peak at 307.0615 represents $M+Na^+$.

5.4.2 Hydroxyzine ($C_{21}H_{27}ClN_2O_2$)

The monoisotopic mass of hydroxyzine is 374.1761 amu.

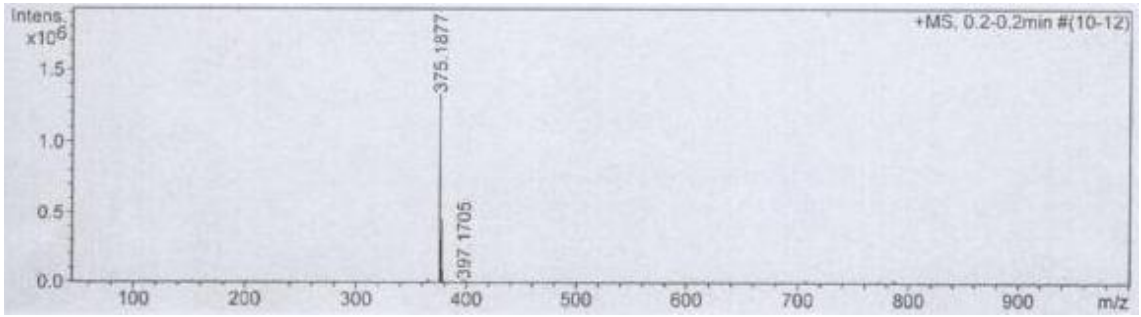

Figure 5.6 ESI mass spectrum of Hydroxyzine tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 375.18, $M+Na^+$ at 397.17, $2M+H^+$ at 749.36 and $2M+Na^+$ at 771.34 (M=monoisotopic mass of hydroxyzine). The isotope distribution for one chlorine atom should be noted, i.e. $M+H^+$: $M+2+H^+$ and $M+Na^+$: $M+2+Na^+$ = 100 : 35.

From **Figure 5.6**, the peak at 375.1877 represents $M+H^+$, 397.1705 represents $M+Na^+$, and 771.3468 represents $2M+Na^+$.

5.4.3 Imipramine ($C_{19}H_{24}N_2$)

The monoisotopic mass of imipramine is 280.1939 amu.

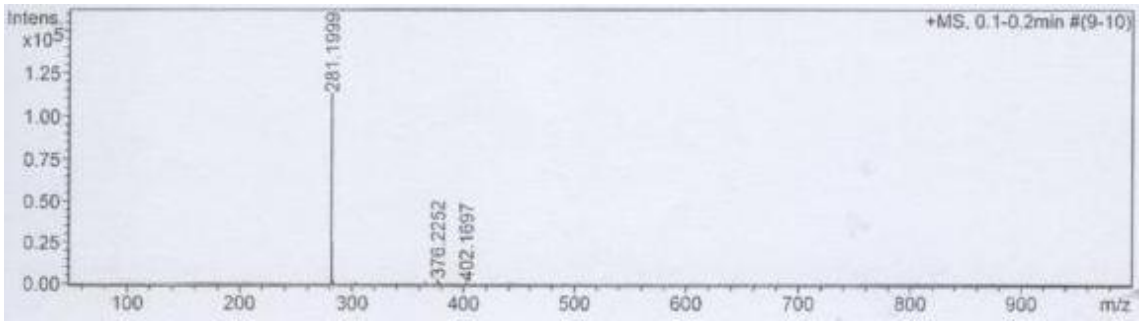

Figure 5.7 ESI mass spectrum of Imipramine tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 281.20, $M+Na^+$ at 303.18, $2M+H^+$ at 561.40 and $2M+Na^+$ at 583.38 (M=monoisotopic mass of imipramine).

From **Figure 5.7**, the peak at 281.1999 represents $M+H^+$, 303.1824 represents $M+Na^+$.

5.4.4 Lorazepam ($C_{15}H_{10}Cl_2N_2O_2$)

The monoisotopic mass of lorazepam is 320.0119 amu.

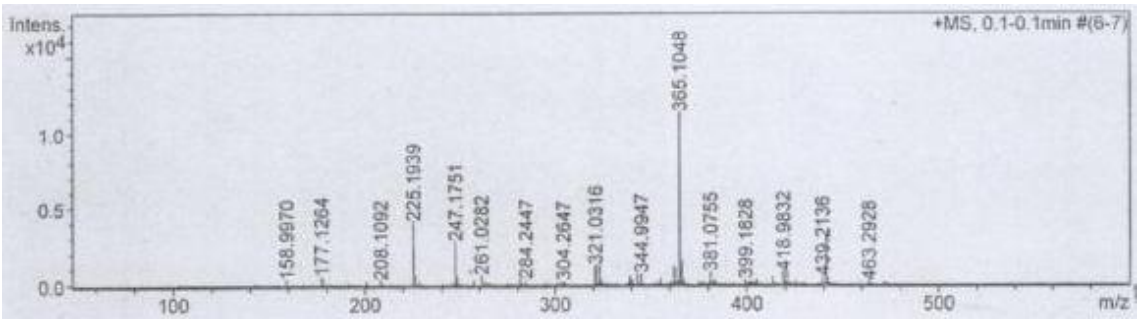

Figure 5.8 ESI mass spectrum of Lorazepam tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 321.02, $M+Na^+$ at 343.00, $2M+H^+$ at 641.03 and $2M+Na^+$ at 663.01 (M=monoisotopic mass of lorazepam). The isotope distribution for two chlorine atoms should be noted, i.e. $M+H^+ : M+2+H^+ : M+4+H^+$ and $M+Na^+ : M+2+Na^+ : M+2+Na^+ = 100 : 65 : 10$.

From **Figure 5.8**, the peak at 321.0316 represents $M+H^+$, 343.0093 represents $M+Na^+$.

5.4.5 Zolpidem ($C_{19}H_{21}N_3O$)

The monoisotopic mass of zolpidem is 307.1685 amu.

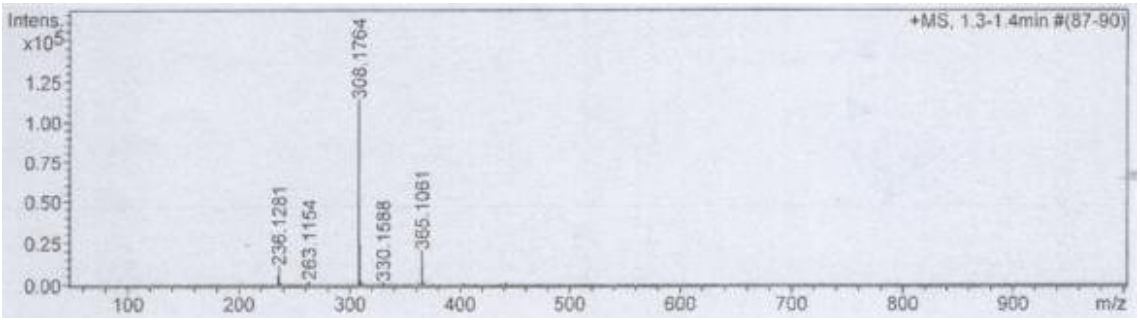

Figure 5.9 ESI mass spectrum of Zolpidem tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 308.18, $M+Na^+$ at 330.16, $2M+H^+$ at 615.34 and $2M+Na^+$ at 637.33 (M=monoisotopic mass of zolpidem).

From **Figure 5.9**, the peak at 308.1764 represents $M+H^+$, 330.1588 represents $M+Na^+$.

5.4.6 Aspirin A ($C_9H_8O_4$)

The monoisotopic mass of aspirin is 180.0423 amu.

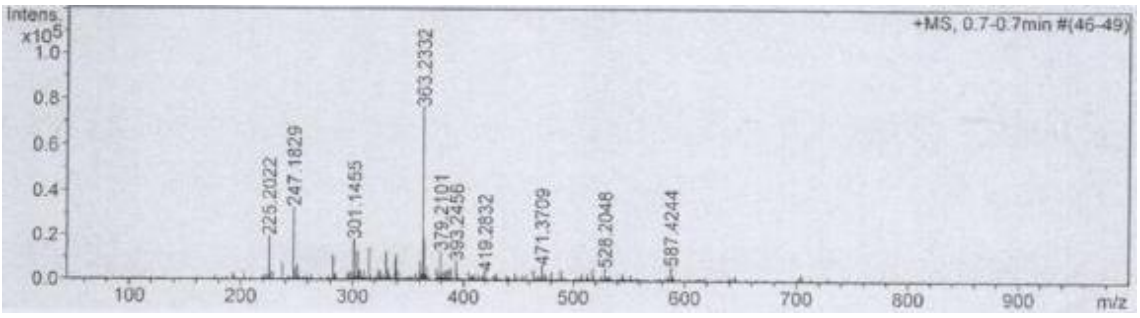

Figure 5.10 ESI mass spectrum of Aspirin A tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 181.05, $M+Na^+$ at 203.03, $2M+H^+$ at 361.09 and $2M+Na^+$ at 383.07 (M=monoisotopic mass of aspirin).

From **Figure 5.10**, there is no peak that represents any of aspirin's adduct.

5.4.7 Aspirin B ($C_9H_8O_4$)

The monoisotopic mass of aspirin is 180.0423 amu.

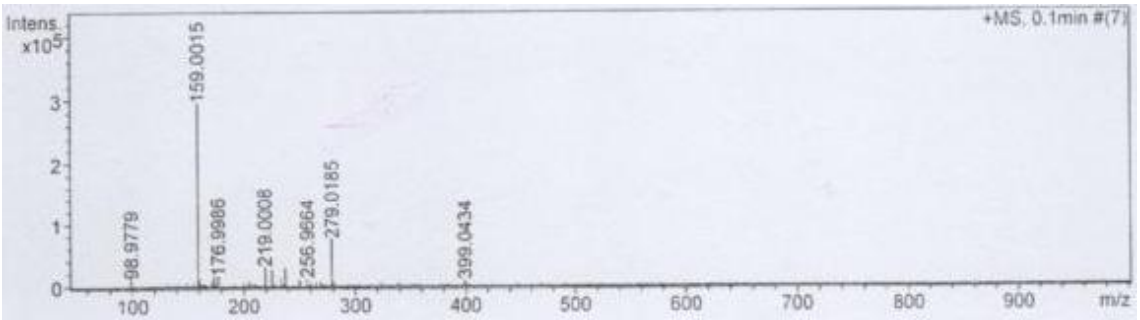

Figure 5.11 ESI mass spectrum of Aspirin B tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 181.05, $M+Na^+$ at 203.03, $2M+H^+$ at 361.09 and $2M+Na^+$ at 383.07 (M=monoisotopic mass of aspirin).

From **Figure 5.11**, the peak at 203.0274 represents $M+Na^+$.

5.4.8 Bromhexine A ($C_{14}H_{20}Br_2N_2$)

The monoisotopic mass of bromhexine is 373.9993 amu.

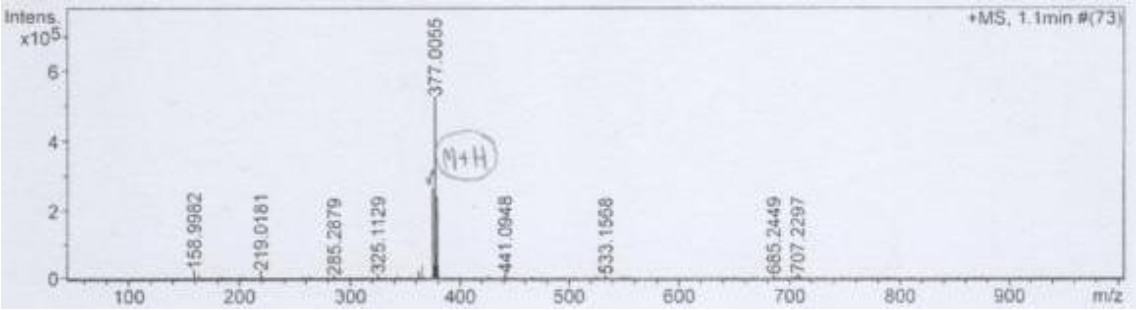

Figure 5.12 ESI mass spectrum of Bromhexine A tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 375.01, $M+Na^+$ at 396.99, $2M+H^+$ at 749.01 and $2M+Na^+$ at 770.99 (M=monoisotopic mass of bromhexine). The isotope distribution for two bromine atoms should be noted, i.e. $M+H^+ : M+2+H^+ : M+4+H^+$ and $M+Na^+ : M+2+Na^+ : M+2+Na^+ = 1 : 2 : 1$.

From **Figure 5.12**, the peak at 375.0080 represents $M+H^+$.

5.4.9 Bromhexine B ($C_{14}H_{20}Br_2N_2$)

The monoisotopic mass of bromhexine is 373.9993 amu.

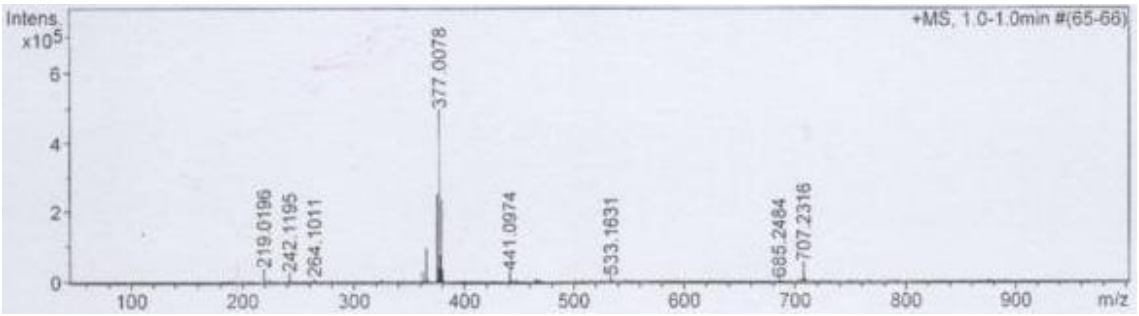

Figure 5.13 ESI mass spectrum of Bromhexine B tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 375.01, $M+Na^+$ at 396.99, $2M+H^+$ at 749.01 and $2M+Na^+$ at 770.99 (M=monoisotopic mass of bromhexine).

From **Figure 5.13**, the peak at 375.0100 represents $M+H^+$.

5.4.10 Ceterizine A ($C_{21}H_{25}ClN_2O_3$)

The monoisotopic mass of ceterizine is 388.1554 amu.

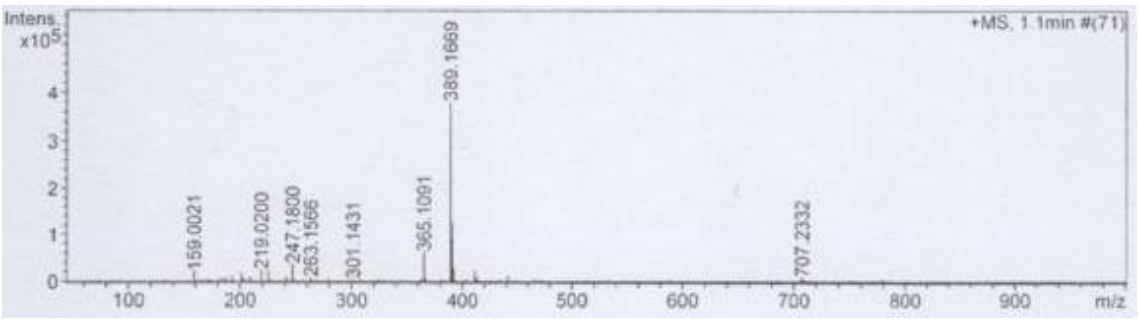

Figure 5.14 ESI mass spectrum of Ceterizine A tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 389.16, $M+Na^+$ at 422.15, $2M+H^+$ at 777.32 and $2M+Na^+$ at 799.30 (M=monoisotopic mass of ceterizine). The isotope distribution for one chlorine atom should be noted, i.e. $M+H^+ : M+2+H^+$ and $M+Na^+ : M+2+Na^+$ = 100 : 35.

From **Figure 5.14**, the peak at 389.1669 represents $M+H^+$, 411.1503 represents $M+Na^+$.

5.4.11 Ceterizine B ($C_{21}H_{25}ClN_2O_3$)

The monoisotopic mass of ceterizine is 388.1554 amu.

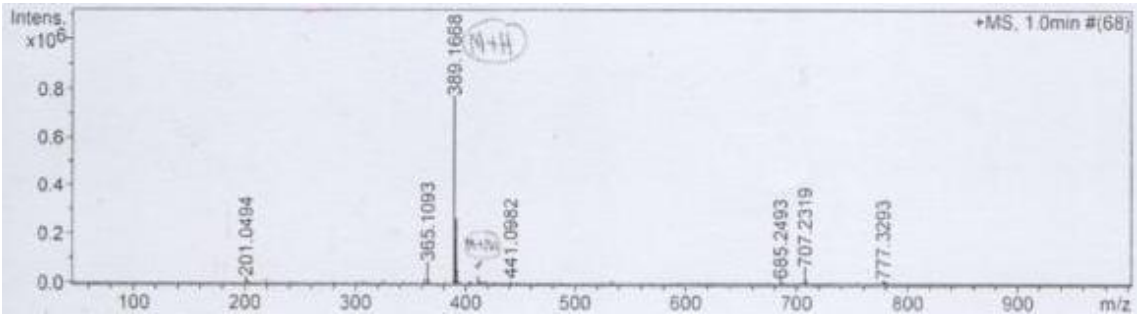

Figure 5.15 ESI mass spectrum of Ceterizine B tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 389.16, $M+Na^+$ at 422.15, $2M+H^+$ at 777.32 and $2M+Na^+$ at 799.30 (M=monoisotopic mass of ceterizine).

From **Figure 5.15**, the peak at 389.1668 represents $M+H^+$, 411.1505 represents $M+Na^+$.

5.4.12 Chlorpheniramine A ($C_{16}H_{19}ClN_2$)

The monoisotopic mass of chlorpheniramine is 274.1237 amu.

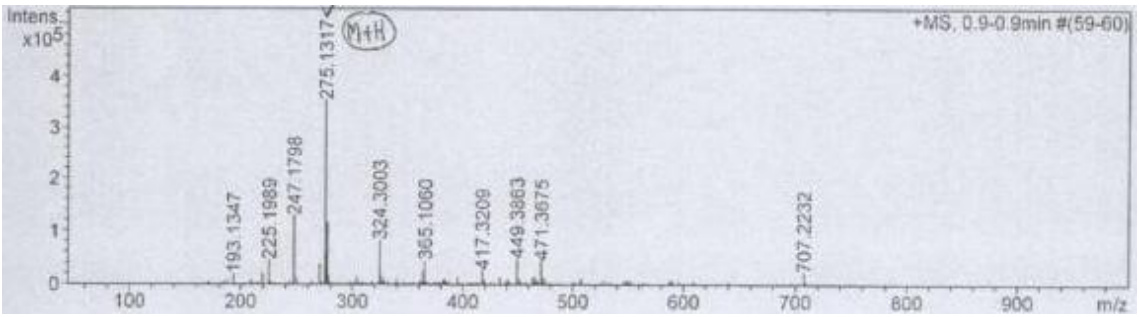

Figure 5.16 ESI mass spectrum of Chlorpheniramine A tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 275.13, $M+Na^+$ at 297.11, $2M+H^+$ at 549.26 and $2M+Na^+$ at 571.24 (M=monoisotopic mass of chlorpheniramine). The isotope

distribution for one chlorine atom should be noted, i.e. $M+H^+$: $M+2+H^+$ and $M+Na^+$: $M+2+Na^+$ = 100 : 35.

From **Figure 5.16**, the peak at 275.1317 represents $M+H^+$.

5.4.13 Chlorpheniramine B ($C_{16}H_{19}ClN_2$)

The monoisotopic mass of chlorpheniramine is 274.1237 amu.

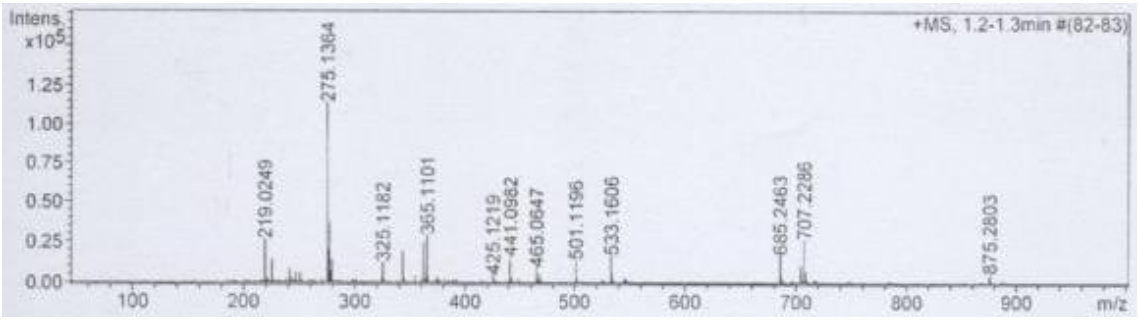

Figure 5.17 ESI mass spectrum of Chlorpheniramine B tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 275.13, $M+Na^+$ at 297.11, $2M+H^+$ at 549.26 and $2M+Na^+$ at 571.24 (M=monoisotopic mass of chlorpheniramine).

From **Figure 5.17**, the peak at 275.1364 represents $M+H^+$.

5.4.14 Mefenamic acid A ($C_{15}H_{15}NO_2$)

The monoisotopic mass of mefenamic acid is 241.1103 amu.

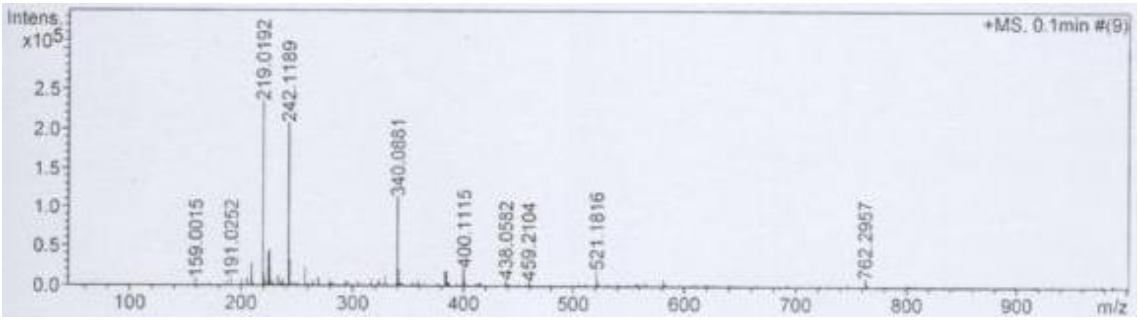

Figure 5.18 ESI mass spectrum of Mefenamic acid A tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 242.12, $M+Na^+$ at 264.10, $2M+H^+$ at 483.23 and $2M+Na^+$ at 505.21 (M=monoisotopic mass of mefenamic acid).

From **Figure 5.18**, the peak at 242.1189 represents $M+H^+$, 264.1003 represents $M+Na^+$.

5.4.15 Mefenamic acid B ($C_{15}H_{15}NO_2$)

The monoisotopic mass of mefenamic acid is 241.1103 amu.

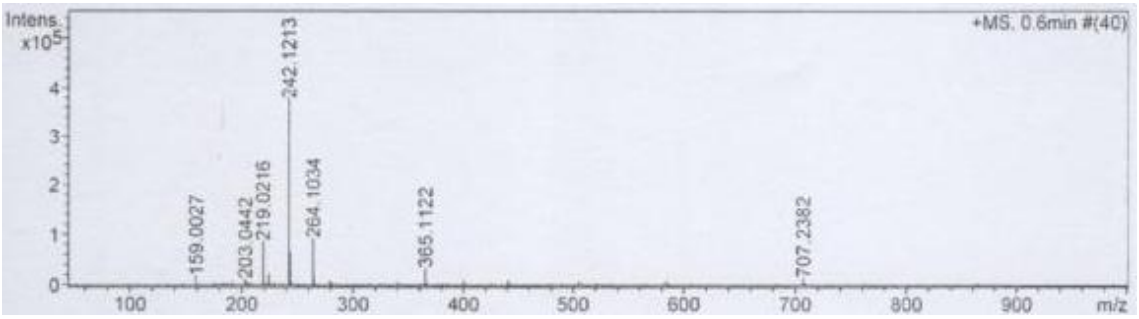

Figure 5.19 ESI mass spectrum of Mefenamic acid B tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 242.12, $M+Na^+$ at 264.10, $2M+H^+$ at 483.23 and $2M+Na^+$ at 505.21 (M=monoisotopic mass of mefenamic acid).

From **Figure 5.19**, the peak at 242.1213 represents $M+H^+$, 264.1034 represents $M+Na^+$.

5.4.16 Omeprazole A ($C_{17}H_{19}N_3O_3S$)

The monoisotopic mass of omeprazole is 345.1147 amu.

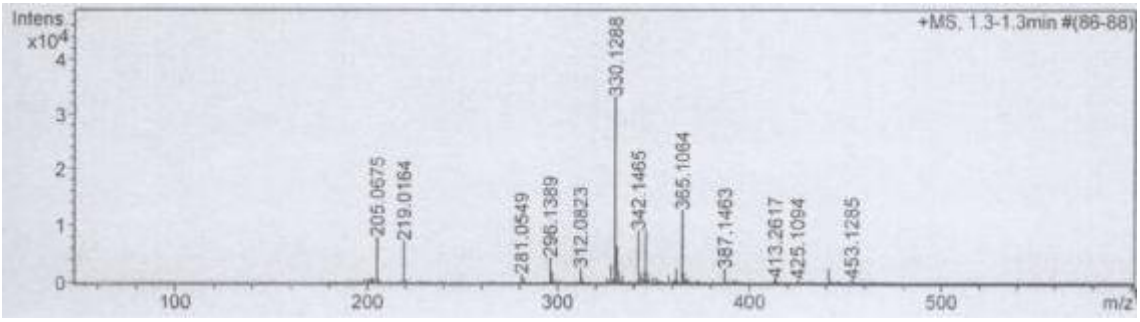

Figure 5.20 ESI mass spectrum of Omeprazole A tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 346.12, $M+Na^+$ at 368.14, $2M+H^+$ at 691.24 and $2M+Na^+$ at 713.22 (M=monoisotopic mass of omeprazole).

From **Figure 5.20**, the peak at 346.1226 represents $M+H^+$, 368.1022 represents $M+Na^+$.

5.4.17 Omeprazole B ($C_{17}H_{19}N_3O_3S$)

The monoisotopic mass of omeprazole is 345.1147 amu.

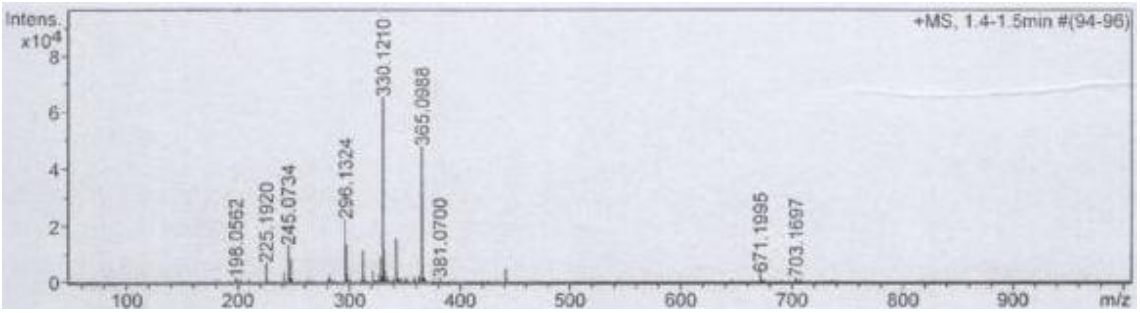

Figure 5.21 ESI mass spectrum of Omeprazole B tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 346.12, $M+Na^+$ at 368.14, $2M+H^+$ at 691.24 and $2M+Na^+$ at 713.22 (M=monoisotopic mass of omeprazole).

From **Figure 5.21**, there is no peak that represents any of omeprazole's adduct.

5.4.18 Roxithromycin A ($C_{41}H_{76}N_2O_{15}$)

The monoisotopic mass of roxithromycin is 836.5246 amu.

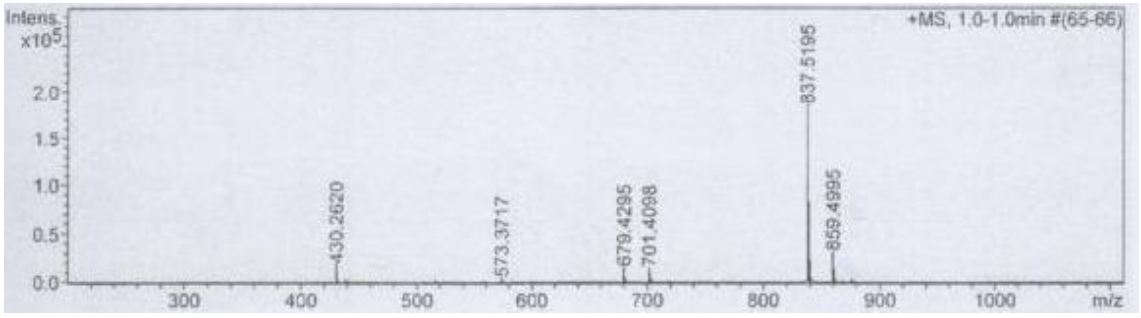

Figure 5.22 ESI mass spectrum of Roxithromycin A tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 837.53, $M+Na^+$ at 859.51, $2M+H^+$ at 1674.06 and $2M+Na^+$ at 1696.04 (M=monoisotopic mass of roxithromycin).

From **Figure 5.22**, the peak at 837.5195 represents $M+H^+$, 859.4995 represents M+Na+.

5.4.19 Roxithromycin B ($C_{41}H_{76}N_2O_{15}$)

The monoisotopic mass of roxithromycin is 836.5246 amu.

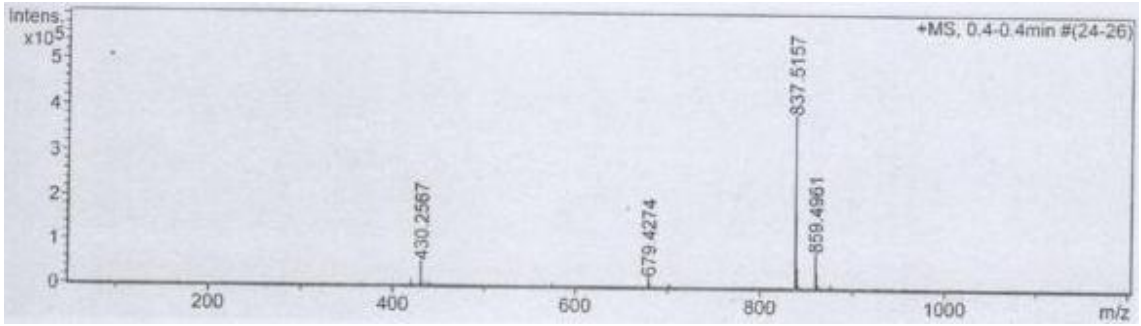

Figure 5.23 ESI mass spectrum of Roxithromycin B tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 837.53, $M+Na^+$ at 859.51, $2M+H^+$ at 1674.06 and $2M+Na^+$ at 1696.04 (M=monoisotopic mass of roxithromycin).

From **Figure 5.23**, the peak at 837.5157 represents $M+H^+$, 859.4961 represents M+Na+.

5.4.20 Amoxicillin A ($C_{16}H_{19}N_3O_5S$)

The monoisotopic mass of amoxicillin is 365.1045 amu.

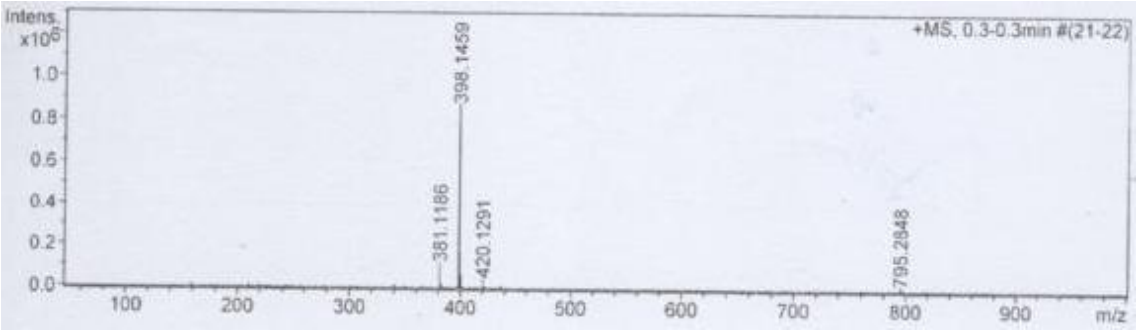

Figure 5.24 ESI mass spectrum of Amoxicillin A tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 366.11, $M+Na^+$ at 388.09, $2M+H^+$ at 731.22 and $2M+Na^+$ at 753.20 (M=monoisotopic mass of amoxicillin).

From **Figure 5.24**, there is no peak that represents any of amoxicillin's adduct.

5.4.21 Amoxicillin B ($C_{16}H_{19}N_3O_5S$)

The monoisotopic mass of amoxicillin is 365.1045 amu.

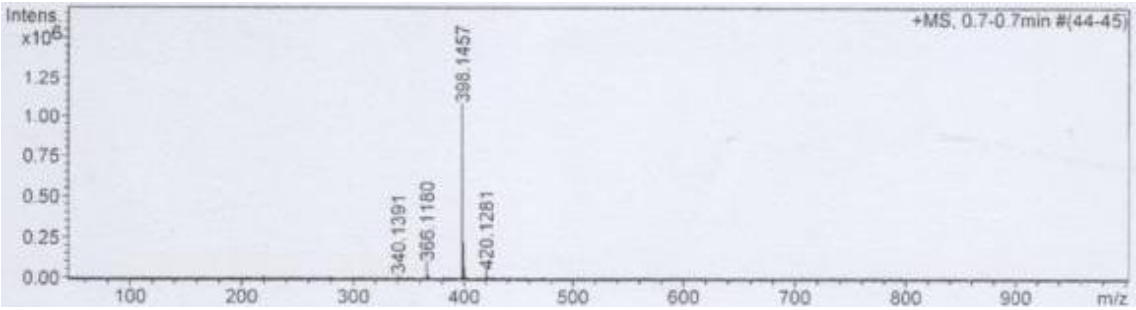

Figure 5.25 ESI mass spectrum of Amoxicillin B tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 366.11, $M+Na^+$ at 388.09, $2M+H^+$ at 731.22 and $2M+Na^+$ at 753.20 (M=monoisotopic mass of amoxicillin).

From **Figure 5.25**, the peak at 366.1180 represents $M+H^+$.

5.4.22 Amoxicillin C ($C_{16}H_{19}N_3O_5S$)

The monoisotopic mass of amoxicillin is 365.1045 amu.

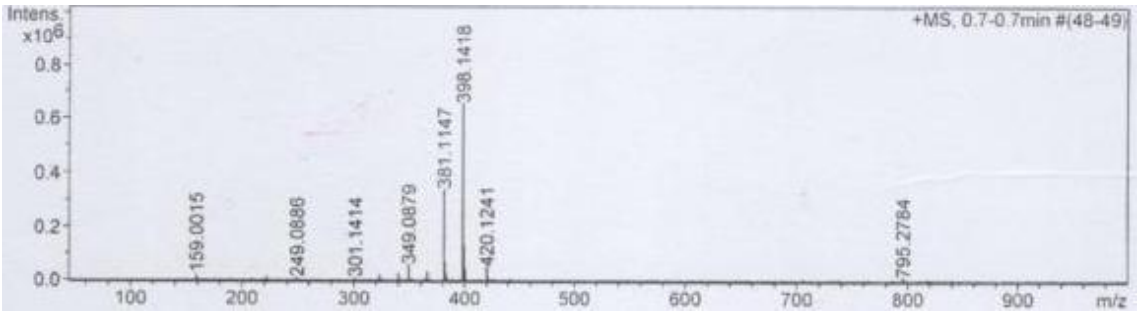

Figure 5.26 ESI mass spectrum of Amoxicillin C tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 366.11, $M+Na^+$ at 388.09, $2M+H^+$ at 731.22 and $2M+Na^+$ at 753.20 (M=monoisotopic mass of amoxicillin).

From **Figure 5.26**, the peak at 366.1145 represents $M+H^+$, 388.0975 represents $M+Na^+$.

5.4.23 Ibuprofen A ($C_{13}H_{18}O_2$)

The monoisotopic mass of ibuprofen is 206.1307 amu.

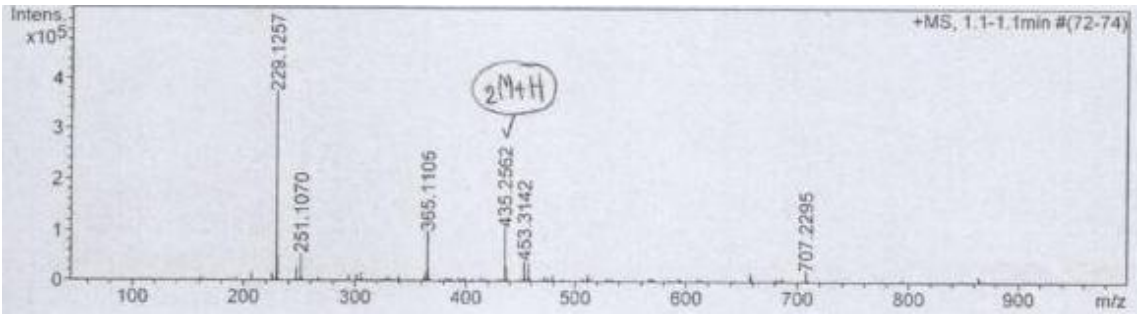

Figure 5.27 ESI mass spectrum of Ibuprofen A tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 207.14, $M+Na^+$ at 229.12, $2M+H^+$ at 413.25 and $2M+Na^+$ at 435.25 (M=monoisotopic mass of ibuprofen).

From **Figure 5.27**, the peak at 229.1275 represents $M+Na^+$, 435.2562 represents $2M+H^+$.

5.4.24 Ibuprofen B ($C_{13}H_{18}O_2$)

The monoisotopic mass of ibuprofen is 206.1307 amu.

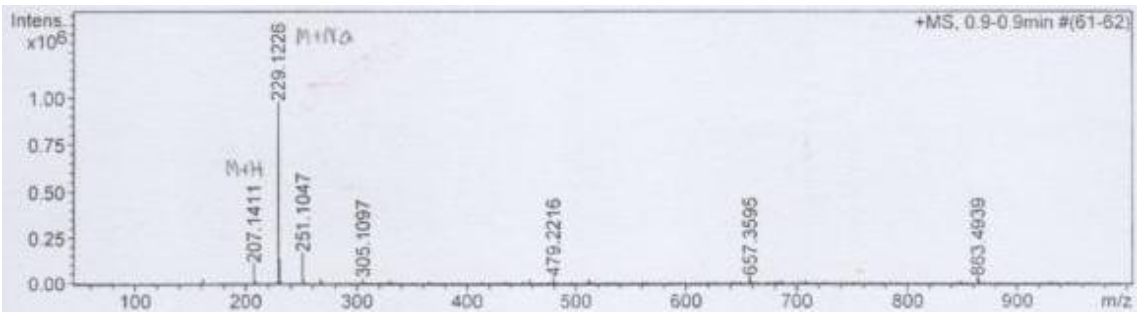

Figure 5.28 ESI mass spectrum of Ibuprofen B tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 207.14, $M+Na^+$ at 229.12, $2M+H^+$ at 413.25 and $2M+Na^+$ at 435.25 (M=monoisotopic mass of ibuprofen).

From **Figure 5.28**, the peak at 207.1411 represents $M+H^+$, 229.1226 represents $M+Na^+$.

5.4.25 Ibuprofen C ($C_{13}H_{18}O_2$)

The monoisotopic mass of ibuprofen is 206.1307 amu.

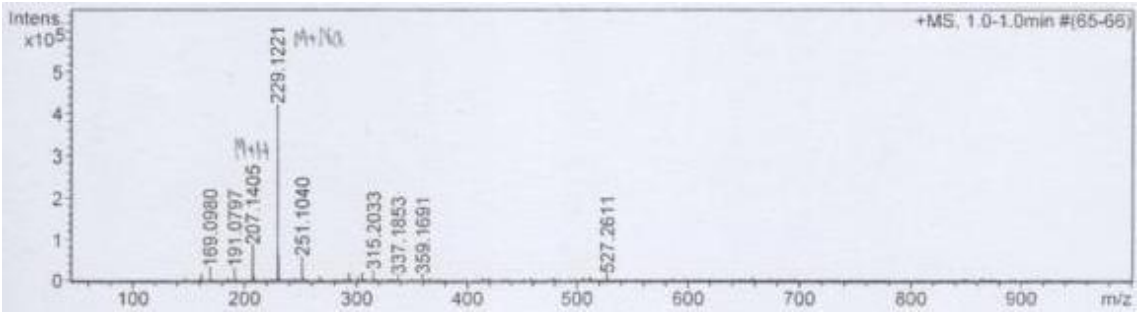

Figure 5.29 ESI mass spectrum of Ibuprofen C tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 207.14, $M+Na^+$ at 229.12, $2M+H^+$ at 413.25 and $2M+Na^+$ at 435.25 (M=monoisotopic mass of ibuprofen).

From **Figure 5.29**, the peak at 207.1405 represents $M+H^+$, 229.1221 represents $M+Na^+$.

5.4.26 Paracetamol A ($C_8H_9NO_2$)

The monoisotopic mass of paracetamol is 151.0633 amu.

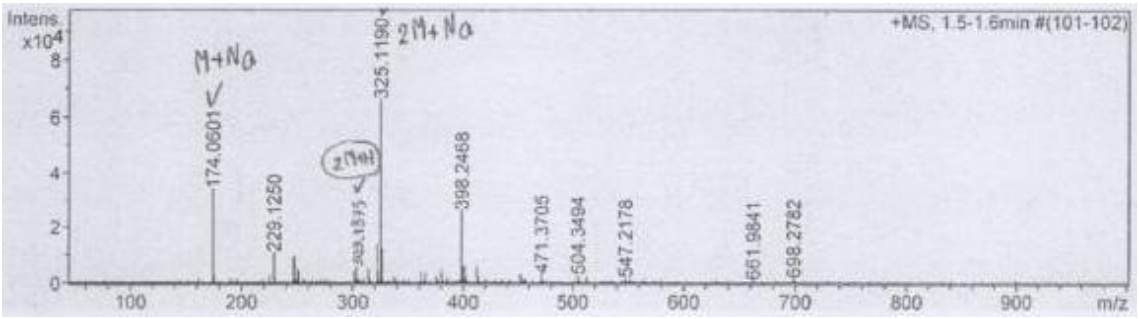

Figure 5.30 ESI mass spectrum of Paracetamol A tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 152.07, $M+Na^+$ at 174.05, $2M+H^+$ at 303.13 and $2M+Na^+$ at 325.12 (M=monoisotopic mass of paracetamol).

From **Figure 5.30**, the peak at 174.0601 represents $M+Na^+$, 303.1375 represents $2M+H^+$ and 325.1190 represents $2M+Na^+$.

5.4.27 Paracetamol B ($C_8H_9NO_2$)

The monoisotopic mass of paracetamol is 151.0633 amu.

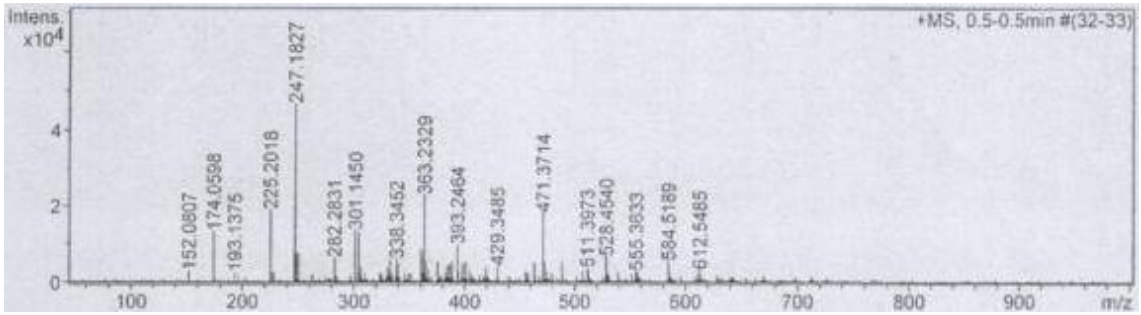

Figure 5.31 ESI mass spectrum of Paracetamol B tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 152.07, $M+Na^+$ at 174.05, $2M+H^+$ at 303.13 and $2M+Na^+$ at 325.12 (M=monoisotopic mass of paracetamol).

From **Figure 5.31**, the peak at 152.0807 represents $M+H^+$, 174.0598 represents $M+Na^+$.

5.4.28 Paracetamol C ($C_8H_9NO_2$)

The monoisotopic mass of paracetamol is 151.0633 amu.

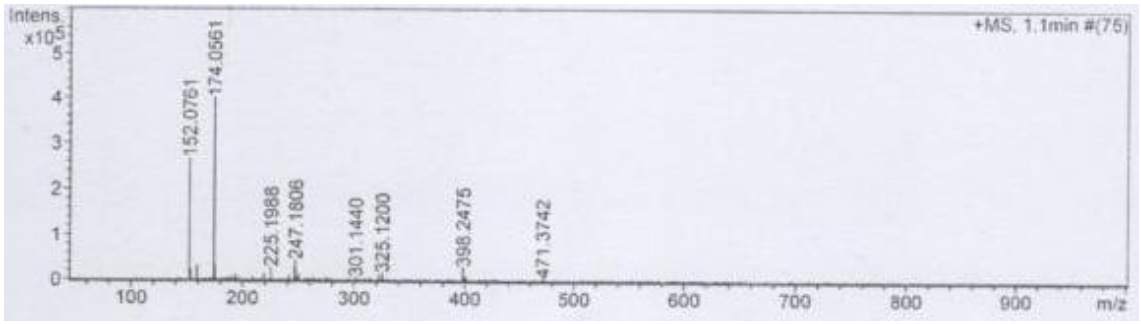

Figure 5.32 ESI mass spectrum of Paracetamol C tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 152.07, $M+Na^+$ at 174.05, $2M+H^+$ at 303.13 and $2M+Na^+$ at 325.12 (M=monoisotopic mass of paracetamol).

From **Figure 5.32**, the peak at 152.0761 represents $M+H^+$, 174.0561 represents $M+Na^+$.

5.4.29 Paracetamol D ($C_8H_9NO_2$)

The monoisotopic mass of paracetamol is 151.0633 amu.

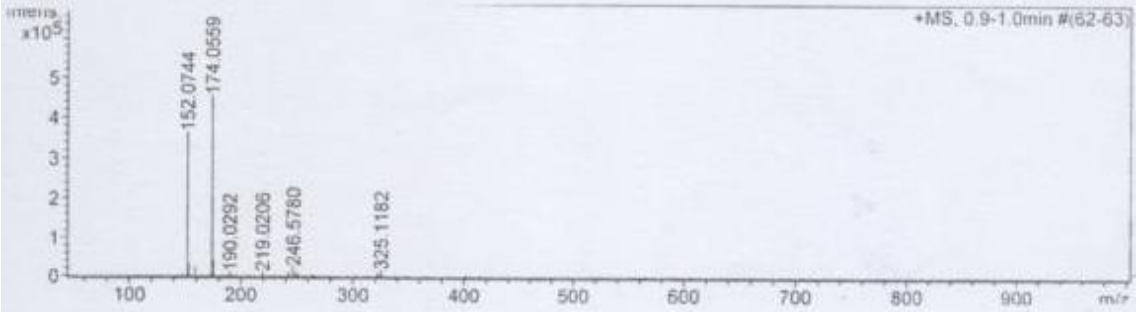

Figure 5.33 ESI mass spectrum of Paracetamol D tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The target peaks are $M+H^+$ at 152.07, $M+Na^+$ at 174.05, $2M+H^+$ at 303.13 and $2M+Na^+$ at 325.12 (M=monoisotopic mass of paracetamol).

From **Figure 5.33**, the peak at 152.0744 represents $M+H^+$, 174.0559 represents $M+Na^+$.

5.4.30 Cold tablet manufacturer A

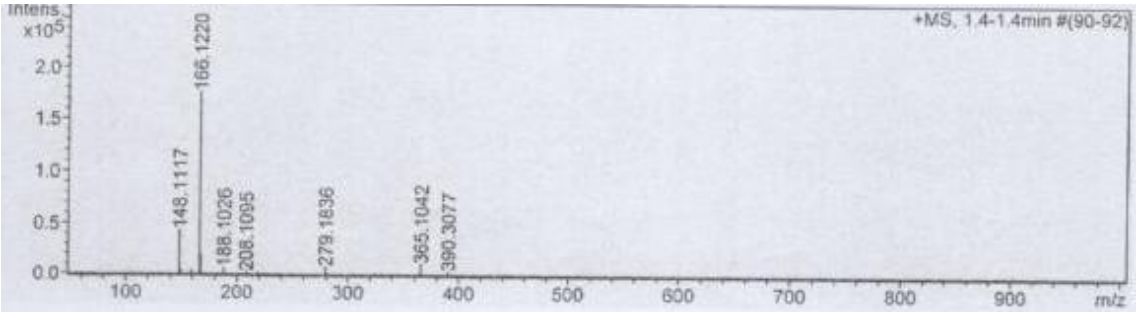

Figure 5.34 ESI mass spectrum of Cold tablet manufacturer A tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The active compounds are pseudoephedrine ($C_{10}H_{15}NO$), and triprolidine ($C_{19}H_{22}N_2$). The monoisotopic masses of pseudoephedrine and triprolidine are 165.1154 and 278.1783 amu., respectively.

The target peaks are M_1+H^+ at 166.12, M_1+Na^+ at 188.11, $2M_1+H^+$ at 331.24 and $2M_1+Na^+$ at 353.22 (M_1=monoisotopic mass of pseudoephedrine), M_2+H^+ at 279.19, M_2+Na^+ at 301.17, $2M_2+H^+$ at 557.36 and $2M_2+Na^+$ at 579.35 (M_2=monoisotopic mass of triprolidine).

From **Figure 5.34**, the peak at 166.1220 represents M_1+H^+, 188.1026 represents M_1+Na^+ and 279.1836 represents M_2+H^+.

5.4.31 Cold tablet manufacturer B

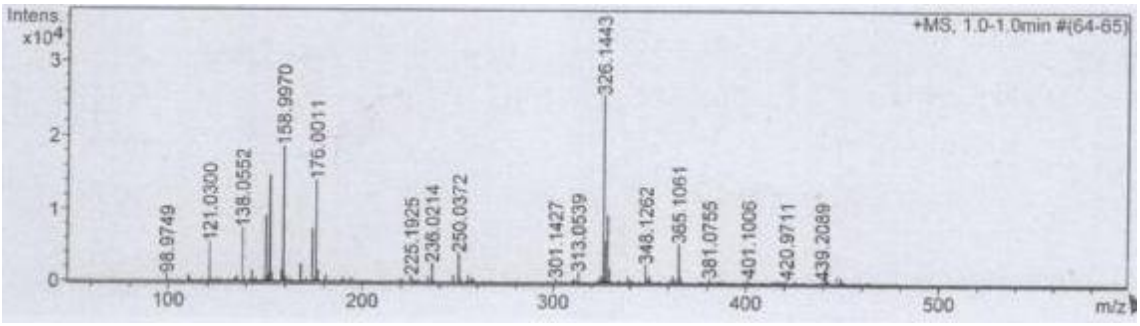

Figure 5.35 ESI mass spectrum of Cold tablet manufacturer B tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The active compounds are paracetamol ($C_8H_9NO_2$), salicylamide ($C_7H_7NO_2$), clemizole ($C_{19}H_{20}ClN_3$) and phenylephrine ($C_8H_{13}NO_2$). The monoisotopic masses of paracetamol, salicylamide, clemizole and phenylephrine are 151.0633, 137.0477, 325.1346 and 155.0946 amu., respectively.

The target peaks are M_1+H^+ at 152.07, M_1+Na^+ at 174.05, $2M_1+H^+$ at 303.13 and $2M_1+Na^+$ at 325.12 (M_1=monoisotopic mass of paracetamol), M_2+H^+ at 138.06, M_2+Na^+ at 160.04, $2M_2+H^+$ at 275.10 and $2M_2+Na^+$ at 279.09 (M_2=monoisotopic mass of salicylamide) M_3+H^+ at 326.14, M_3+Na^+ at 348.12, $2M_3+H^+$ at 651.28 and $2M_3+Na^+$ at 673.26 (M_3=monoisotopic mass of clemizole and the isotope distribution for one chlorine atom should be noted, i.e. $M+H^+$: $M+2+H^+$ and $M+Na^+$: $M+2+Na^+$ = 100 : 35.), M_4+H^+ at 156.10, M_4+Na^+ at 178.08, $2M_4+H^+$ at 311.20 and $2M_4+Na^+$ at 333.18 (M_2=monoisotopic mass of phenylephrine).

From **Figure 5.35**, the peak at 138.0552 represents M_2+H^+, 152.0711 represents M_1+H^+, 174.0519 represents M_1+Na^+, 325.1189 represents $2M_1+Na^+$, 326.1443 represents M_3+H^+ and 348.1262 represents M_3+Na^+.

5.4.32 Migraine headache tablet

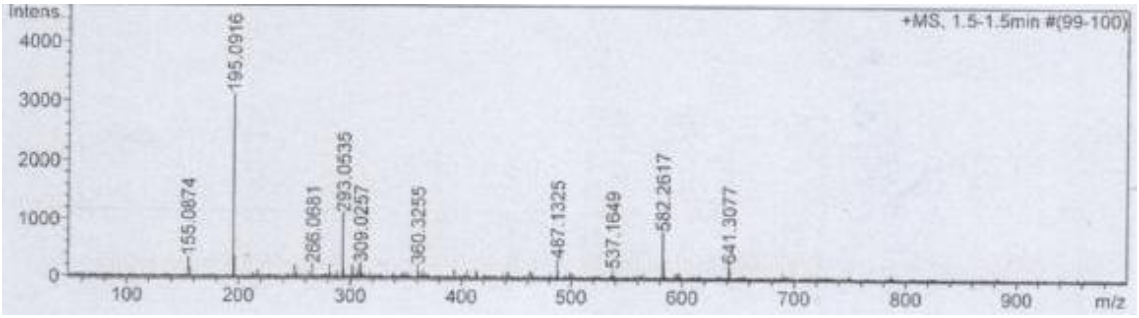

Figure 5.36 ESI mass spectrum of Migraine headache tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The active compounds are caffeine ($C_8H_{10}N_4O_2$), and ergotamine ($C_{33}H_{35}N_5O_5$). The monoisotopic masses of caffeine and ergotamine are 194.0804 and 581.2638 amu., respectively.

The target peaks are M_1+H^+ at 195.09, M_1+Na^+ at 217.07, $2M_1+H^+$ at 389.17 and $2M_1+Na^+$ at 411.15 (M_1=monoisotopic mass of caffeine), M_2+H^+ at 582.27, M_2+Na^+ at 604.25, $2M_2+H^+$ at 1163.54 and $2M_2+Na^+$ at 1185.52 (M_2=monoisotopic mass of ergotamine).

From **Figure 5.36**, the peak at 195.0916 represents M_1+H^+ and 582.2617 represents M_2+H^+.

5.4.33 Muscle relaxant tablet

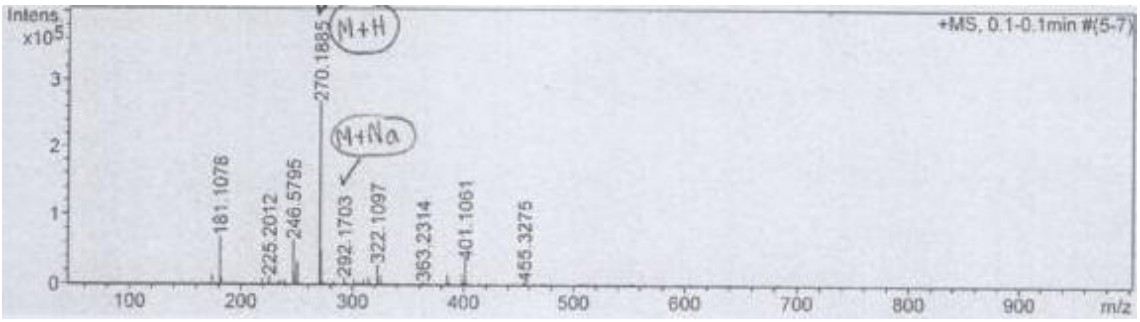

Figure 5.37 ESI mass spectrum of Muscle relaxant tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The active compounds are paracetamol ($C_8H_{19}NO_2$), and orphenadrine ($C_{18}H_{23}NO$). The monoisotopic masses of paracetamol and orphenadrine are 151.0633 and 269.1780 amu., respectively.

The target peaks are M_1+H^+ at 152.07, M_1+Na^+ at 174.05, $2M_1+H^+$ at 303.13 and $2M_1+Na^+$ at 325.12 (M_1=monoisotopic mass of paracetamol), M_2+H^+ at 270.19, M_2+Na^+ at 292.17, $2M_2+H^+$ at 539.36 and $2M_2+Na^+$ at 561.35 (M_2=monoisotopic mass of orphenadrine).

From **Figure 5.37**, the peak at 174.0596 represents M_1+Na^+, 270.1885 represents M_2+H^+, 292.1703 represents M_2+Na^+ and 325.1188 represents $2M_1+Na^+$.

5.4.34 Cold tablet manufacturer C

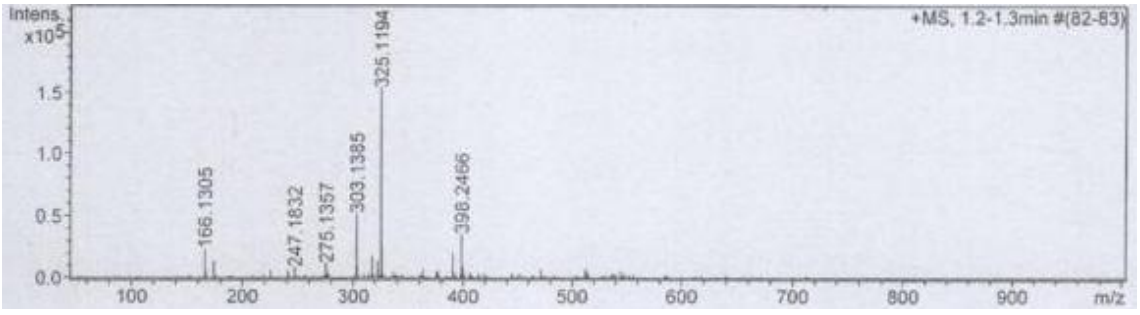

Figure 5.38 ESI mass spectrum of Cold tablet manufacturer C tablet sample (100 ppm in 80:20 (v/v), MeOH:1% acetic acid)

The active compounds are paracetamol ($C_8H_9NO_2$), pseudoephedrine ($C_{10}H_{15}NO$) and chlorpheniramine ($C_{16}H_{19}ClN_2$). The monoisotopic masses of

paracetamol, pseudoephedrine and chlorpheniramine are 151.0633, 165.1154, and 274.1237 amu., respectively.

The target peaks are M_1+H^+ at 152.07, M_1+Na^+ at 174.05, $2M_1+H^+$ at 303.13 and $2M_1+Na^+$ at 325.12 (M_1=monoisotopic mass of paracetamol), M_2+H^+ at 166.12, M_2+Na^+ at 188.11, $2M_2+H^+$ at 331.24 and $2M_2+Na^+$ at 353.22 (M_2=monoisotopic mass of pseudoephedrine) M_3+H^+ at 275.13, M_3+Na^+ at 297.11, $2M_3+H^+$ at 549.26 and $2M_3+Na^+$ at 571.24 (M_3=monoisotopic mass of chlorpheniramine).

From **Figure 5.38**, the peak at 166.1305 represents M_2+H^+, 174.0602 represents M_1+Na^+, 275.1357 represents M_3+H^+, 303.1385 represents $2M_1+H^+$, and 325.1194 represents $2M_1+Na^+$.

5.5 Tablet Identification Procedure

For identifying an unknown tablet sample from the ESI mass spectra of the tablet, the following procedure is proposed.

Step 1 – Prepare the unknown tablet sample as 100 ppm solution in 80:20 (v/v), MeOH:1% acetic acid.
Step 2 – Obtain the sample's spectrum using ESI in positive mode.
Step 3 – Compare m/z of the base peak in the spectrum to the Compound Target List.
Step 4 – If there is no match with the base peak, then select the next abundant peak.
Step 5 – Repeat step 3 and 4.

The *Compound Target List* (**Table 5.1**) is the calculated monoisotopic mass of adducts of common drugs that can form from the ionization process. For any compound four adducts are often found to form in the positive ion mode electrospray. There are $M+H^+$, $M+Na^+$, $2M+H^+$ and $2M+Na^+$ (M=monoisotopic mass of the neutral compound). Consequently, the Compound Target List contains *m/z* value of four adducts for each compound.

Table 5.1 Compound Target List in increasing order of *m/z* value of cationic adduct

m/z	**Ionized Species**
138.06	Salicylamide+H^+
152.07	Paracetamol+H^+
156.10	Phenylephrine+H^+
160.04	Salicylamide+Na^+
166.12	Pseudoephedrine+H^+
174.05	Paracetamol+Na^+
178.08	Phenylephrine+Na^+
181.05	Aspirin+H^+
188.11	Pseudoephedrine+Na^+
195.09	Caffeine+H^+
203.03	Aspirin+Na^+
207.14	Ibuprofen+H^+
217.07	Caffeine+Na^+
229.12	Ibuprofen+Na^+
242.12	Mefenamic+H^+
264.1	Mefenamic+Na^+
270.19	Orphenadrine+H^+
275.10	2Salicylamide+H^+
275.13	Chlorpheniramine+H^+ *
279.19	Triprolidine+H^+
281.20	Imipramine+H^+
285.08	Diazepam+H^+ *
292.17	Orphenadrine+Na^+
297.09	2Salicylamide+Na^+
297.11	Chlorpheniramine+Na^+ *
301.17	Triprolidine+Na^+
303.13	2Paracetamol+H^+
303.18	Imipramine+Na^+
307.06	Diazepam+Na^+ *

Table 5.1 Compound Target List in increasing order of *m/z* value of cationic adduct (cont.)

m/z	**Ionized Species**
308.18	Zolpidem+H^+
311.20	2Phenylephrine+H^+
321.02	Lorazepam+H^+ **
325.12	2Paracetamol+Na^+
326.14	Clemizole+H^+ *
330.16	Zolpidem+Na^+
331.24	2Pseudoephedrine+H^+
333.18	2Phenylephrine+Na^+
343.00	Lorazepam+Na^+ **
346.12	Omeprazole+H^+
348.12	Clemizole+Na^+ *
353.22	2Pseudoephedrine+Na^+
361.09	2Aspirin+H^+
366.11	Amoxicillin+H^+
368.14	Omeprazole+Na^+
375.01	Bromhexine+H^+ ***
375.18	Hydroxyzine+H^+ *
383.07	2Aspirin+Na^+
388.09	Amoxicillin+Na^+
389.16	Ceterizine+H^+ *
389.17	2Caffeine+H^+
396.99	Bromhexine+Na^+ ***
397.17	Hydroxyzine+Na^+ *
411.15	Ceterizine+Na^+ *
411.15	2Caffeine+Na^+
413.27	2Ibuprofen+H^+
435.25	2Ibuprofen+Na^+
483.23	2Mefenamic+H^+

Table 5.1 Compound Target List in increasing order of *m/z* value of cationic adduct (cont.)

m/z	**Ionized Species**
505.21	2Mefenamic+Na^+
539.36	2Orphenadrine+H^+
549.26	2Chlorpheniramine+H^+ *
557.36	2Triprolidine+H^+
561.35	2Orphenadrine+Na^+
561.40	2Imipramine+H^+
569.15	2Diazepam+H^+ *
571.24	2Chlorpheniramine+Na^+ *
579.35	2Triprolidine+Na^+
582.27	Ergotamine+H^+
583.38	2Imipramine+Na^+
591.13	2Diazepam+Na^+ *
604.25	Ergotamine+Na^+
615.34	2Zolpidem+H^+
637.33	2Zolpidem+Na^+
641.03	2Lorazepam+H^+ **
651.28	2Clemizole+H^+ *
663.01	2Lorazepam+Na^+ **
673.26	2Clemizole+Na^+ *
691.24	2Omeprazole+H^+
713.22	2Omeprazole+Na^+
731.22	2Amoxicillin+H^+
749.01	2Bromhexine+H^+ ***
749.36	2Hydroxyzine+H^+ *
753.20	2Amoxicillin+Na^+
770.99	2Bromhexine+Na^+ ***
771.34	2Hydroxyzine+Na^+ *
777.32	2Ceterizine+H^+ *

Table 5.1 Compound Target List in increasing order of *m/z* value of cationic adduct (cont.)

m/z	**Ionized Species**
799.30	2Ceterizine+Na^+ *
837.53	Roxithromycin+H^+
859.51	Roxithromycin+Na^+
1163.54	2Ergotamine+H^+
1185.52	2Ergotamine+Na^+
1674.06	2Roxithromycin+H^+
1696.04	2Roxithromycin+Na^+

* Note : isotopic mass pattern for one chlorine atom
** Note : isotopic mass pattern for two chlorine atoms
*** Note : isotopic mass pattern for two bromine atoms

Firstly, the *m/z* of base peak compared with the Compound Target List. If its *m/z* value (±0.05) is equal to a value from the list, it indicates that the tablet sample contains that compound.

If the base peak did not match any value in the list, then the next highest peak is compared with the list. Again, if its *m/z* value (±0.05) is equal to a value from the list, it indicates that the tablet sample contains that compound.

The comparison is continued for all the peaks having signal-to-noise ratio more than 50.

More than one peak could be matched to the values in the target list, due to the fact that; more than one type of adducts can be formed for one compound: or the sample contains more than one compound.

If no peak matched with the list, it means that either the sample did not contain the compound in the list, or no adducts was produced due to ion suppression effect of the matrix in the tablet.

5.6 The identification

The Tablet Identification Procedure using the Compound Target List was applied to 34 samples. It was found that 22 samples (or 65%) were easily identified,

due to the fact that the base peak is the compound's target peak; 9 samples (or 26%) were identified, using characteristic peaks that were not the base peak; and 3 samples (9%) could not be identified, as no peaks could be matched the *m/z* value in the Compound Target List.

Table 5.2 Sample classification according to ability in identification with ESI-HRTOF-MS technique

Samples easily identified	**Samples identified**	**Samples not identified**
Diazepam	Lorazepam	Aspirin A
Hydroxyzine	Aspirin B	Omeprazole B
Imipramine	Bromhexine A	Amoxicillin A
Zolpidem	Bromhexine B	
Ceterizine A	Mefenamic acid A	
Ceterizine B	Omeprazole A	
Chlorpheniramine A	Amoxicillin B	
Chlorpheniramine B	Amoxicillin C	
Mefenamic acid B	Paracetamol B	
Roxithromycin A		
Roxithromycin B		
Ibuprofen A		
Ibuprofen B		
Ibuprofen C		
Paracetamol A		
Paracetamol C		
Paracetamol D		
Cold tablet manufacturer A		
Cold tablet manufacturer B		
Migraine headache tablet		
Muscle relaxant tablet		
Cold tablet manufacturer C		

Note: A, B, C and D indicate different manufacturer.

CHAPTER VI
CONCLUSION

Tablet identification by Direct Injection Electrospray Ionization High Resolution Time of Flight Mass Spectrometry is found to be a convenient method for tablet identification. However this technique was unable to identify all the tablet samples and may not be suitable screening technique.

However, injecting the sample solution (5-10 μl) onto a reversed-phase C18 column and following the chromatogram using ESI-MS detection should overcome the ion-suppression effect and also only the proton-adduct need to be monitored. Selections of suitable column and mobile-phase have to be developed.

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APPENDIX

TABLE OF ISOTOPIC ABUNDANCE AND EXACT MASS

Element	Mass Number	Relative Abundance	Exact Mass
^{1}H	1	99.985	1.007825
^{2}H	2	0.015	2.014102
^{12}C	12	98.90	12(by definition)
^{13}C	13	1.10	13.003355
^{14}N	14	99.63	14.003074
^{15}N	15	0.37	15.000109
^{16}O	16	99.76	15.994915
^{17}O	17	0.04	16.999132
^{18}O	18	0.20	17.999160
^{23}Na	23	100	22.989769
^{32}S	32	95.02	31.972071
^{33}S	33	0.75	32.971459
^{34}S	34	4.21	33.967867
^{36}S	36	0.02	35.967081
^{35}Cl	35	75.77	34.968852
^{37}Cl	37	24.23	36.965903
^{79}Br	79	50.69	78.918337
^{81}Br	81	49.31	80.916290

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