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Analysis of Synthetic Drugs in Adulterated Chinese Medicine by High Performance Liquid Chromatography/Electrospray Mass Spectrometry

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ABSTRACT

High performance liquid chromatography / ultraviolet (HPLC / UV) coupled to electrospray ionization (ESI) mass spectrometry (MS) provides a highly reliable method for the qualitative analysis of synthetic chemical drugs in adulterated traditional Chinese medicine. Electrospray is most suited to compounds of high polarity or thermolabile because the ionization is performed in solution and vaporization of analyte before ionization is not necessary. The dubious adulterants were identified by their retention times, molecular ions and specific fragment ions produced from the in source collisional induced dissociation.

Key words: **high performance liquid chromatography, electrospray, in source collisional induced dissociation.**

Tradition Chinese medicines are sometimes illegally adulterated by the addition of synthetic chemical drugs. Conventional analytical method involves the separation of the adulterants by Thin-Layer Chromatography (TLC) following identification by retarding factor and /or ultraviolet spectroscopy. These methods are in general time consuming, labor extensive and more importantly lack the specificity for high confidence identification.

Recently, methods based on high performance liquid chromatography (HPLC) and capillary electrophoresis have been developed for the analysis of synthetic drugs in tradition Chinese medicine[(1~6)]. Although these methods are superior to conventional TLC method in time of analysis and resolving power, due to the use of UV or diode array detectors, the specificity is still not adequate for absolute identification.

Mass spectrometry is known for its high sensitivity and high specificity. In general, very high specificity can be achieved with minimal quantity of samples. The combination of mass spectrometry and chromatography proved to be a powerful method to identify the dubious compounds in complicated mixture because absolute identification is often obtained with retention time, molecule ion and many structural characteristic fragment ions present in the mass spectra. Since its development in 60s, gas chromatography / mass

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spectrometry (GC / MS) has been proved to be a very powerful analytical technique. Unfortunately, without chemical derivatization, this technique is limited to volatile and thermally stable compounds. During recent years, HPLC/MS methods based on atmospheric pressure ionization (API) has received considerable attention for the analysis of nonvolatile and thermally labile compounds. Thereupon LC / MS is widely used in the fields of biology, biochemistry, environmental science, pharmaceutical chemistry and analytical chemistry.

The most popular API interface is electrospray ionization. At electrospray interface, the mobile phase passes through a metal capillary (outer diameter is 0.2mm, inner diameter is 0.1mm) which is maintained between 2 and 4 kV with respect to the ion source. As a result of the high voltage, charge separation is formed. Positive ions drift toward the liquid surface and negative ions drift away from the liquid surface. If too many positive ions gather on the liquid surface, it results in instability on the liquid surface and the Taylor cone is formed (Figure 1)[7]. When the voltage is high enough, charged aerosols are formed. These charged droplets evaporate during flight and the charge density on the surface of droplets increases to such an extent that the charge repulsion exceeds the surface tension-a "Coulomb explosion" occurs [8] Smaller droplets with higher mass to charge ratio are formed. This sequence of events repeats until the radius of curvature of a droplet becomes small enough that the field due to the surface charge is strong enough to desorb ions directly from the droplet into the gas phase [7,9].

For electrospray ionization, the analyte molecules must be present in ionized form (as preformed ion) in solution. ESI is just a process of ejecting the preformed ion from liquid phase to gas phase. Therefore, in comparison with other ionization methods, ESI produces ions with a minimum of internal energy and thus little or no fragment ions are observed (Figure 2a). In order to obtain more structural information about the

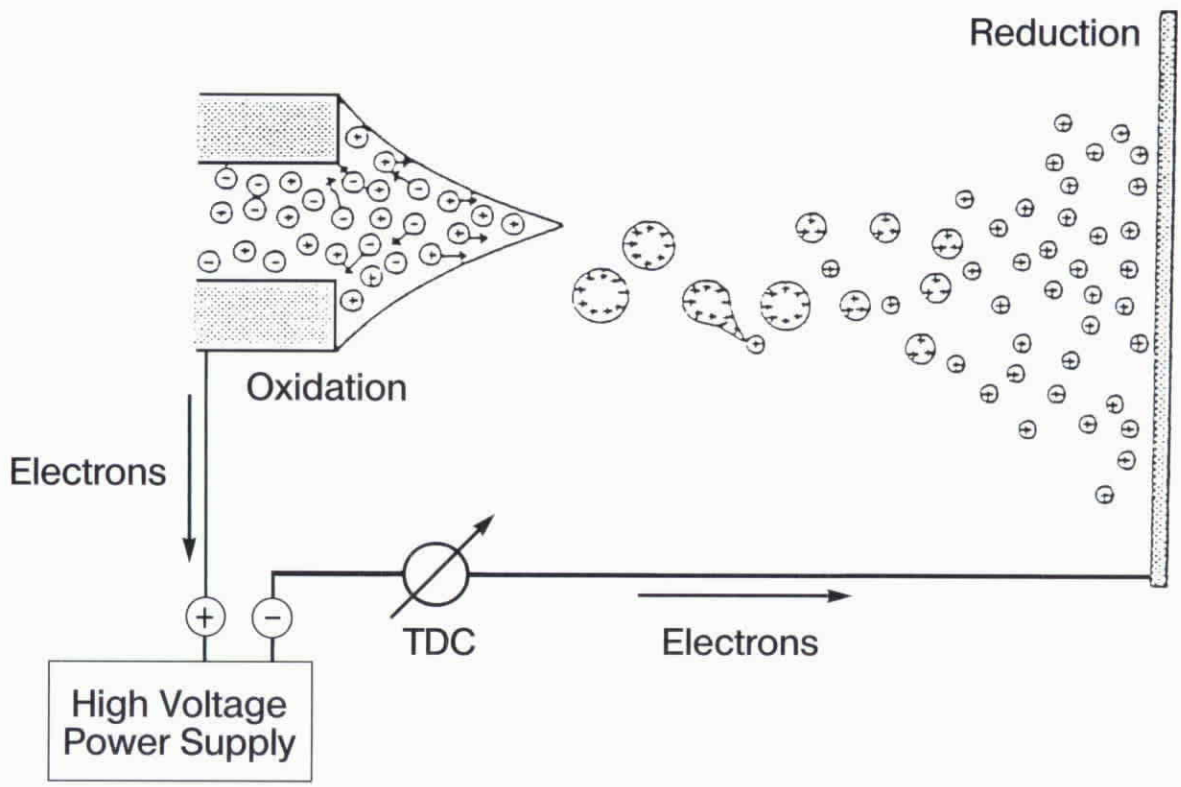

Figure 1. Charged droplets formation at the capillary tip.

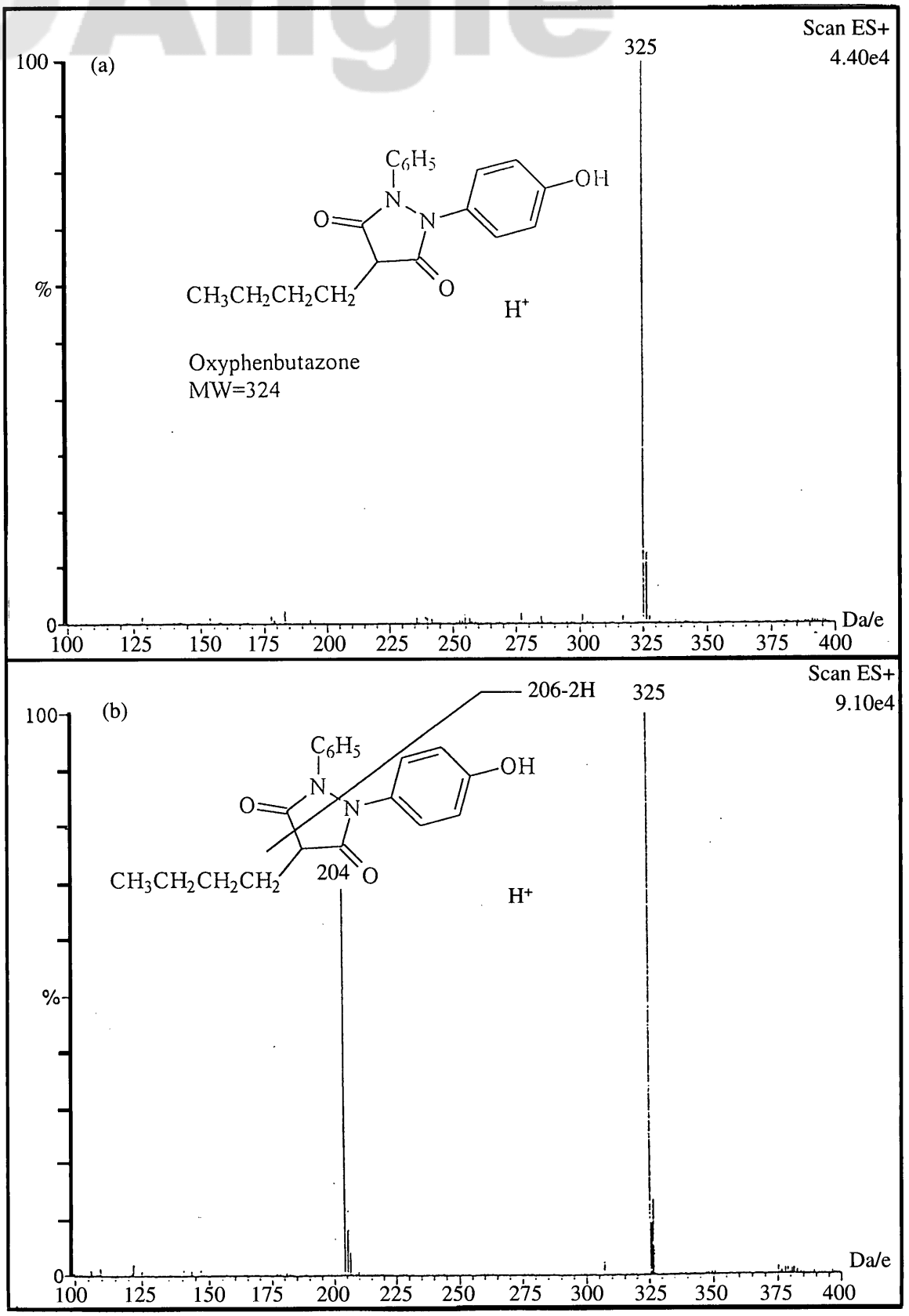

Figure 2. Electrospray mass spectrum of oxyphenbutazone (a) at soft ionization condition (b) at in source CID condition.

analyte, in source collisional induced dissociation (in source CID) is used. In the in source CID, a potential difference is applied between the sample cone and the skimmer (Figure 3). When the molecular ions pass through the sample cone, they are accelerated by the potential difference. These fast moving ions are then collided with gas molecules and the reliability of identification is further enhanced with the production of many structurally specific fragment ions (Figure 2b).

HPLC / ESI-MS was applied to the analysis of adulterants in traditional Chinese medicine. A microbore column(GSK ODS-2, 5μm, 1mm × 15mm) was chosen as the separation column because it possessed higher separation efficiency, better mass sensitivity, smaller sample injection volume and lower mobile phase flow rate. The lower mobile phase flow rate made it possible that HPLC could be combined directly with mass spectrometer without post column splitting [10-12]. The HPLC was coupled with an ultraviolet (UV) detector before connecting to ESI-MS (Figure 4).

Several adulterated Chinese medicines were analyzed using HPLC / UV / ESI-MS. The samples were extracted with 10ml of ethanol and 1μl of the extract was injected directly into the HPLC / MS. To improve the confidence of identification, in addition to the protonated molecule ion, a major fragment ion resulting from the ion source CID was also selected for the reconstructed mass chromatograms. The result of one sample is shown in Figure 5. Acetaminophen, bucetin,

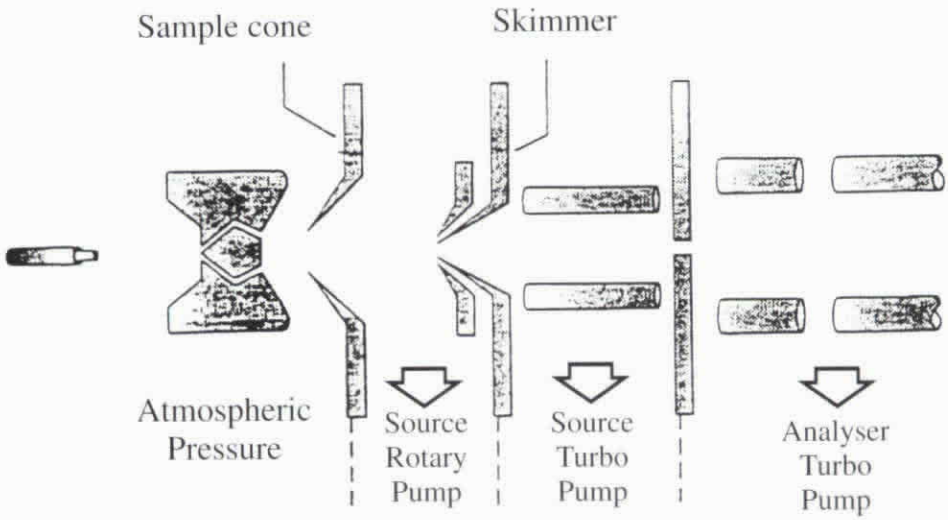

Figure 3. Schematic diagram of electrospray interface.

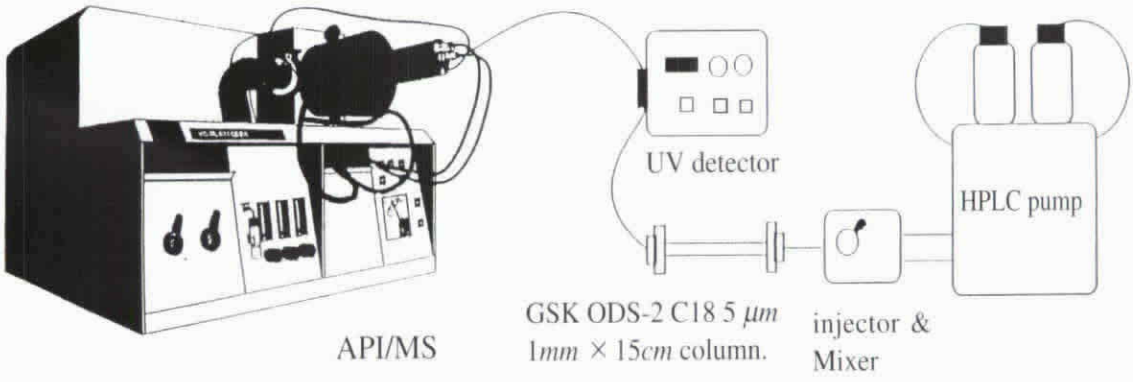

Figure 4. Schematic diagram of HPLC/UV/ESI-MS.

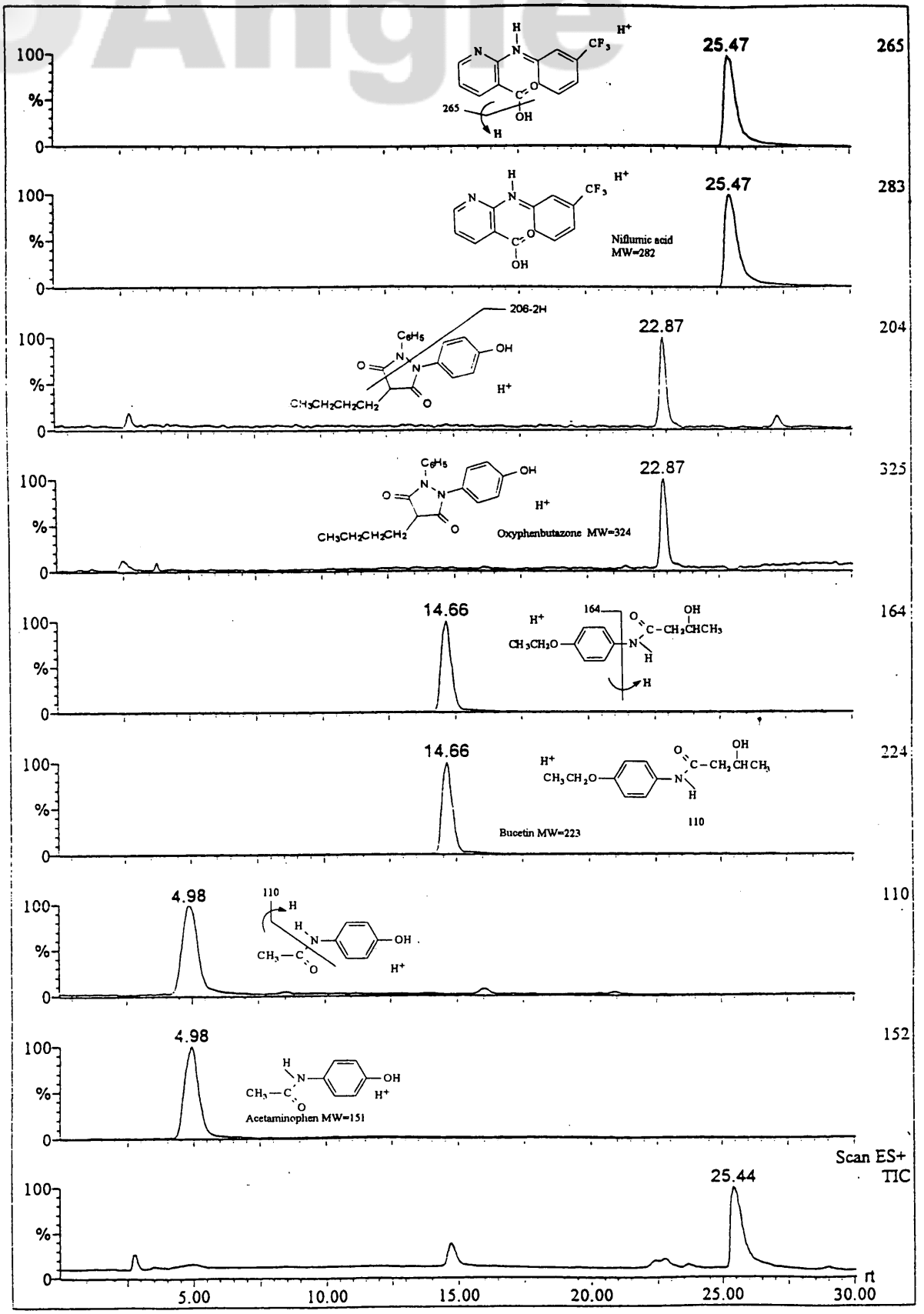

Figure 5. The mass chromatograms of an adulterated Chinese medicine.

oxyphenbutazone, and niflumic acid were detected in this sample. The m/z 152 and m/z 110 ions at 4.98 minutes correspond to the protonated molecular ion and the loss of CH_2CO from the protonated molecular ion of acetaminophen (MW 151). The m/z 224 and m/z 164 ions at 14.66 minutes correspond to the protoned molecular ion and the loss of $CH_3CH(OH)CH_3$ from the protonated molecular ion of bucetin (MW 223). The m/z 325 and 204 ions correspond to the protonated molecular ion and a ring cleavage fragment ion (as shown in Figure 5) of oxyphenbutazone (MW 324). The m/z 283 and 265 ions correspond to the protonated molecular ion and a loss of water molecule from the protonated molecular ion of niflumic acid (MW 282).

The example shown in Figure 5 demonstrated that the synthetic chemical drugs in adulterated Chinese medicine can be easily analyzed by HPLC / ESI-MS without derivatization as often required in GC / MS analysis. Very high confidence of qualitative identification can be achieved with this approach. It is highly unlikely that there is another compound having the same molecular weight, same fragment ion and same retention time as the target compound.

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以高效液相層析質譜儀分析中藥中摻加之西藥

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摘　要

高效率液相層析 / 紫外光可見光偵測器與電灑法質譜儀相銜接 (HPLC / UV / ESI-MS) 對於中藥中所摻加的合成西藥提供了一個高可信度的分析方法。電灑法離子化過程在溶液中進行，樣品離子化前不需先熱揮發成氣態分子，因此適合高極性、熱不穩定樣品。可疑的西藥添加物經由其滯留時間的對照、分子離子以及利用離子化室內碰撞引致裂解法 (in source CID) 所產生的特徵碎片離子得到高可信度的鑑定。

關鍵詞：高效率液相層析，電灑法，離子化室內碰撞引致裂解。