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**Determination of Urinary Clopamide by Using LC/APCI/MS**

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**Abstract** The paper describes a LC/APCI/MS application for the analysis of clopamide(CL). The structure of CL is thermal unstable due to the amido bond in the molecule. The decomposed products in GC analysis were discussed multiple peaks and decrease the sensitivity of detection. The decomposition was avoided and gave single peak with high sensitivity by using LC/APCI/MS. The conditions of APCI were optimized. The excretion and the detection limit were also described.

**Introduction**

Clopamide [3-(Aminosulfonyl)-4-chrolo-N-(2,6-dimethyl-1-piperidinyl) benzamide, CL] is a diuretic with properties similar to those of the thiazide diuretics even though it does not contain a thiazide ring system. diuresis is initiated in 1 to 2 hours, reaches a maximum in about 3 to 6 hours, and lasts for up to 24 hours. It has been given in the treatment of oedema or hypertension[1]. It was banned by IOC in 1997.

ESI and APCI have different strengths and weaknesses and generally they complement each other. ESI is best suited to the analysis of moderately to highly polar molecules. It works well for large, biological molecules and pharmaceuticals, especially molecules that ionize in solution and exhibit multiple charging. It also works for small molecules as long as they are fairly polar. APCI is not as well suited for the analysis of large, multiple-charged molecules. It works best for the analysis of smaller, non-polar or moderately polar molecules.

The misuses of diuretics is monitored by LC[2] or GC/MS[3-7]. The structure of Clopamide is shown in Figure 1. This structure is thermal unstable due to the amido bond in the molecule, which can decompose in gas chromatographic injector or during the process of derivatization then results in multiple peaks and decreases the sensitivity of detection. The paper describes a LC/APCI/MS application for the analysis of CL. The decomposition was avoided by using LC/APCI/MS and gave single peak with high sensitivity.

**Experimental**

**Instruments and experimental conditions**

LC/MS: HP 1090 HPLC-HP5989 MS, HP G1075A APCI interface

HPLC column: C-18 reversed-phase column, 5µm, 2.1mm×15cm (Zorbax, USA)
Mobile phase: A: acetic acid solution 1%, B: acetonitrile
Gradient: 0-10 min: B= 0-20%, 10-20min: B=20-70%, flow rate: 0.4ml/min.
Tune maker: a mixture of valine, tri-valine and hexa-tyrosine(HP company), target ions: m/z118.08, m/z508.20, m/z 997.39, CID(collision-induced dissociation) setting: CapEx=150V, electron-Multiplier voltage:1800V, drying nitrogen flow: 8000ml/min, Drying nitrogen temperature: 350°C, nebulizing nitrogen pressure: 80psi. GC/MS: MD800 GC/MS system (Fison), HP-5 column (25m×0.2mm×0.33 μm), splitless mode. The oven temperature was: 100°C, rate1: 20°C/min to 180°C, rate2: 5°C/min to 220°C, and rate3: 20°C/min to 280°C and maintained for 10 min. Injector: 250°C. Interface: 290°C. The carrier gas was helium with the rate of 0.98 ml/min. The mass spectra were obtained in full scan mode from m/z 40 to m/z 400.

Chemicals and reagents
CL was obtained from Sigma. All solvents and reagents used in experiment were chromatographic grade (Beijing Chemicals Company).

Drug administration and urine collection
40mg of CL was orally taken by a healthy volunteer. The urine was collected till 48h.

Extraction
To 3ml of urine 0.5g solid buffer (NaHCO₃/K₂CO₃, 3/2), 4ml diethyl ether were added. After being shaken for 20min and centrifuged for 10min (2500rpm) the organic layer was separated and evaporated to dryness in a stream of nitrogen. The residual was derivatized for GC/MS or redissolved in 200μl of methanol for LC/MS analysis.

Derivatization
To the residue, 100μl of acetone, 100μl of iodomethane(redistilled) and 50mg of potassium carbonate(dehydrated at 300°C for 1 hour) were added. The mixture was heated at 70°C for 1 hour. 2μl of the solution was injected into GC/MS.

Results and discussion
The products in GC/MS analysis
From the positive urine, after the chemical treatment as described above, three peaks could be obtained in the total ion chromatogram. The corresponding mass spectra and fragment pattern of the three products were shown in fig.1,2 and 3. That suggested CL was not suitable to GC analysis.
LC/APCI/MS analysis

From the positive urine, after the chemical treatment as described above (without derivatization), single peak for CL could be obtained in the total ion chromatogram (fig 4). That was good for the determination. The corresponding mass spectrum was also shown in fig 4.

Detection limit and linearity range

After a series concentrations of CL standard was analyzed, the detection limit for CL in scan mode with single m/z 346 was 3 ng. The concentration of CL was proportional to the response from 5 ng to 10 μg.

Urinary Excretion Profiles of CL

With the method presented here, urinary excretion profiles of CL was showed in Fig 5. The CL reached the top concentration at 12 h. The CL in original form was detectable in 24 hr after oral administration. No metabolite was observed in the experiment.

Optimum of HPLC flow rate

To compare to electrospray, APCI is easier to match the flow rate of HPLC. For electrospray normally required a flow rate less than 100 μl/min whereas APCI more than 100 μl/min to get a high-enough ionization efficiency. According to the results of the experiments (Fig 6), the abundance in peak height of required signal was increased by 17.5 times as the flow rate increased from 100 μl/min to 400 μl/min. At the flow rate of 400 μl/min for a column of diameter 2.0 mm, HPLC column could obtain a good separation simultaneously.

CID voltage

In APCI/MS procedure, to get fragmentation of a molecule, CID (collision induced dissociation) will be performed instead of electronic impact. The collision energy can be changed by different setting of the voltage applied on the capillary exit (CapEx). In the CID test on CL, significant changes in the relative abundances of molecular ion and other fragments could be observed by altering the voltage. As shown in Fig. 7, the optimal value for the determination is 150 V.

Conclusion

The labile compound, CL which can decompose in gas chromatographic injector or during the process of derivatization then results in multiple peaks and decreases the sensitivity of detection, was determined by LC/APCI/MS. The decomposition was avoided and gave single peak with high sensitivity. Suitable to the determination.
References


Fig.1 EI mass spectrum of dimethylated clopamide

Fig.2 Characteristic ion fragmentation of clopamide trimethylated derivative (I)
Fig 3 El mass spectrum of clopamide trimethylated derivative (II)

Fig. 4 TIC and APCI Mass spectrum of clopamide

Fig 5 Excretion curve of clopamide in the volunteer’s urine

Fig. 6 The effect of column flow

Fig. 7 Effect of CID voltage on peak height of clopamide