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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 excration 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 ($NaHCO_3/K_2CO_3$, 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 5ng to 10μg.

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 12h. 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 100μl/min whereas APCI more than 100μl/min to get a high-enough ionization efficiency. According to the results of the experiments (Fig 6), the aboundance in peak height of required signal was increased by 17.5 times as the flow rate increased from 100μl/min to 400μl/min. At the flow rate of 400μl/min for a column of diameter 2.0mm, 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 150V.

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.

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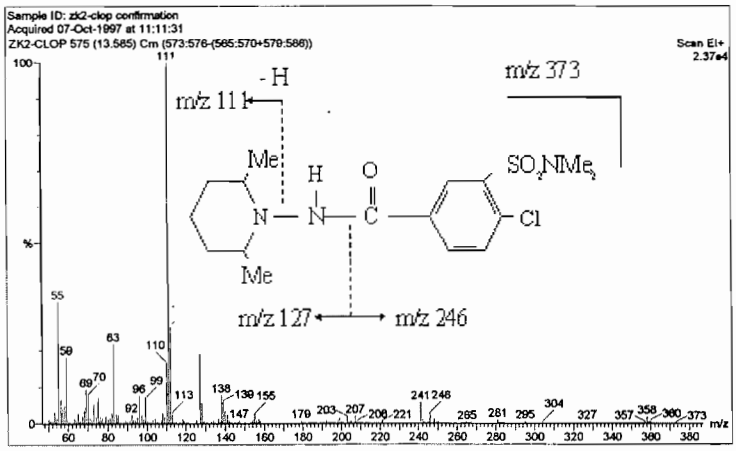

Fig.1 EI mass spectrum of dimethylated clopamide

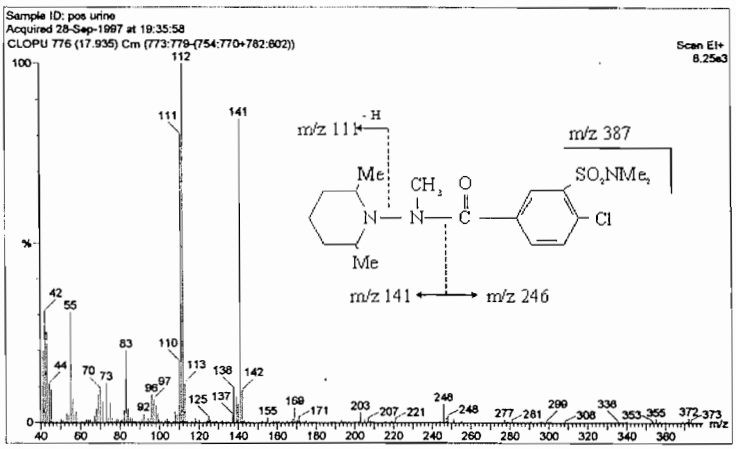

Fig 2 Characteristic ion fragmentation of clopamide trimethylated derivative (I)

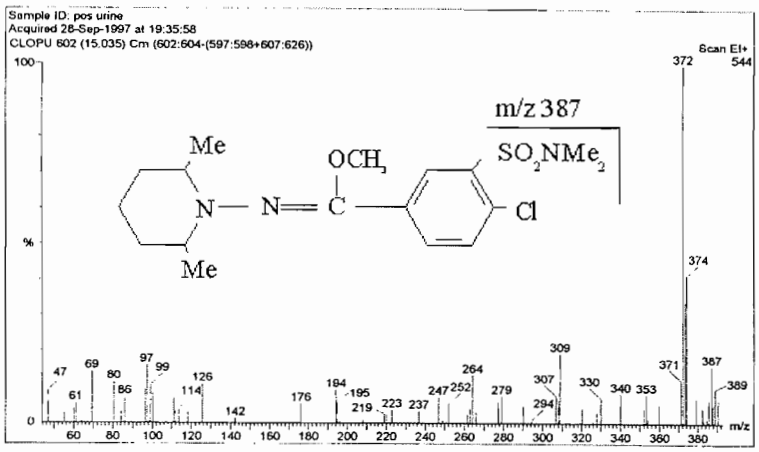

Fig3 EI mass spectrum of clopamide trimethylated derivative (II)

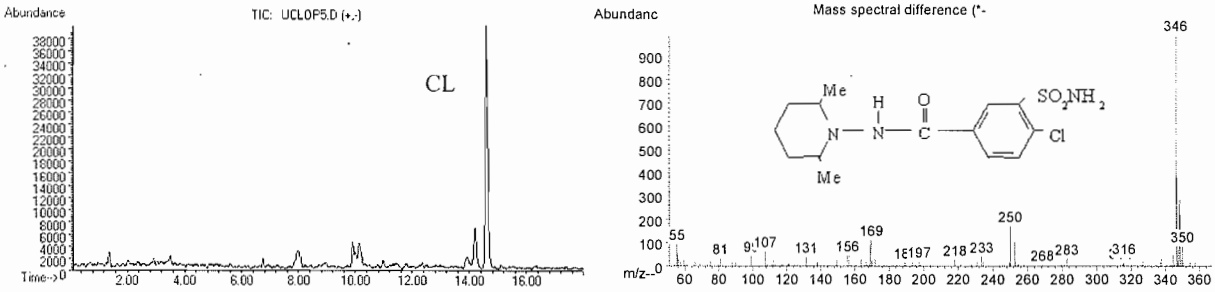

Fig. 4 TIC and APCI Mass spectrum of clopamide

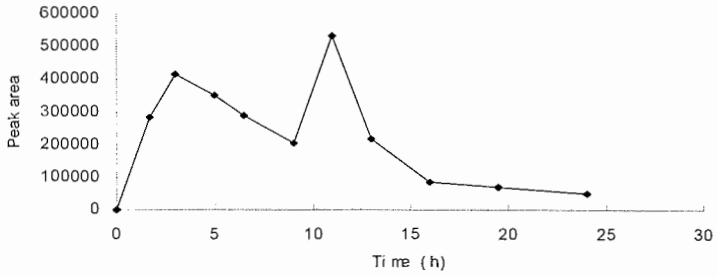

Fig 5 Excretion curve of clopamide in the volunteer's urine

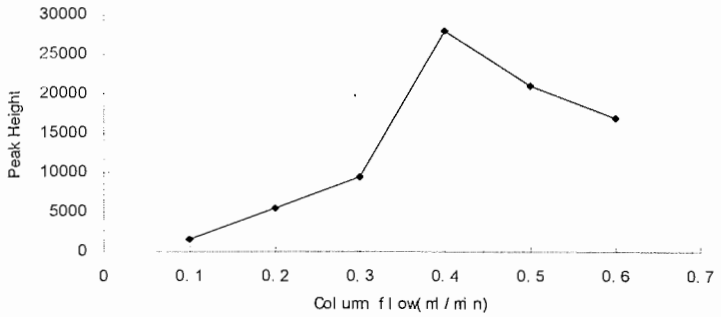

Fig.6 The effect of column flow

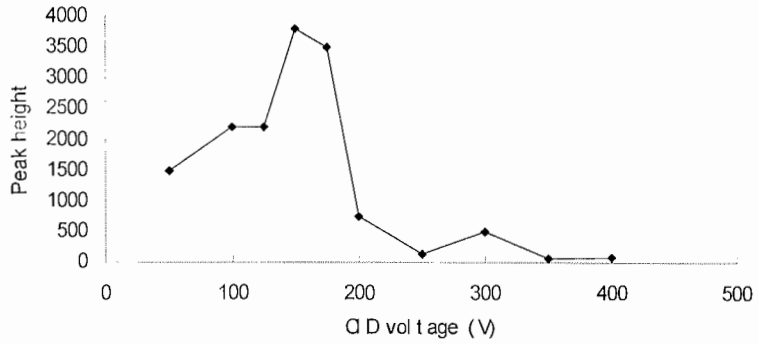

Fig.7 Effect of CID voltage on peak height of clopamide