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原　　報

LIQUID CHROMATOGRAPHIC / MASS SPECTROMETRIC DETERMINATION OF MORPHINE, CODEINE AND COCAINE IN HUMAN SERA USING A NEW INTERNAL SURFACE REVERSED PHASE COLUMN

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新内面逆相ODSカラムを用いた血清中モルヒネ，コデインならびにコカインのLC/MSによる定量

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Summary

A new internal surface reversed phase column (Shim-pack MAYI-ODS) was used for analysis of morphine, codeine and cocaine in human sera by LC/MS/MS. The column enabled direct injection of crude biological samples without pretreatment realizing a rapid analytical procedure. The recoveries of morphine, codeine and cocaine spiked into human sera were 50−60 %. The regression equations for morphine, codeine and cocaine in sera, using atropine as internal standard, showed good linearity in the ranges of 100−500, 50−250 and 20−100 ng/ml, respectively; the detection limits were 50, 20 and 10 ng/ml, respectively. The coefficients of intra-day variation for the three compounds spiked into sera were less than 7 %. The proposed method seems useful for toxicological analysis of three drugs, because of rapidness and reproducibility.

Key words: Morphine; Codeine; Cocaine; Atropine; New internal surface reversed phase column; Shim-pack MAYI-ODS; LC/MS/MS

Introduction

Liquid chromatography (LC) / mass spectrometry (MS) and LC/MS/MS are now popular tools for detection and identification of abused drugs in human specimens [1-3]. Before such analysis, the crude specimens are usually subjected to clean-up by liquid-liquid extraction or solid-phase extraction [1].

Recently, a new LC column, Shodex MSpak GF-310, has been developed; it covers elimination of large molecules such as proteins and nucleic acids together with usual column separation of small molecules. The column enables crude biological samples to be directly applied to the column without any pretreatment for LC, LC/MS and LC/MS/MS. In our recent studies, we have successfully used this column for LC/MS analysis of haloperidol and its metabolites in human samples [4,5]. In this report, we present another unique type of columns, Shim-pack MAYI-ODS, for LC/MS/MS analysis of morphine, codeine and cocaine in human sera.

Experimental

Materials

Morphine hydrochloride, codeine phosphate and cocaine hydrochloride were obtained from Shionogi & Co., Ltd.(Osaka); atropine sulfate from Sigma Chemical Co. (St. Louis, MO, USA). Other common chemicals used were of the highest purity commercially available.

Sera were separated by centrifugation (3,000 rpm, 5 min) from whole blood of healthy subjects.

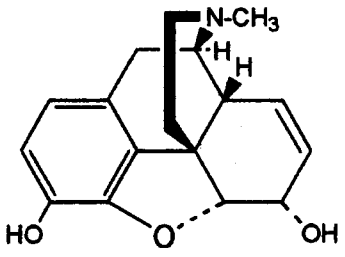
LC/MS(/MS)

The LC, used in connection with MS (/MS), was made on an HP 1100 Series instrument (Agilent Technologies, Palo Alto, CA, USA). The LC column used for chromatographic separation was Shim-pack MAYI-ODS (10 x 4.6 mm i.d., Shimadzu, Kyoto). The elution gradient was 0 % B (100 % A, 5-min hold) to 80 % B (20 % A) over 25 min at a flow rate of 0.2 ml/min; solvent A was distilled water containing 7.5 mM ammonium acetate, and solvent B acetonitrile containing 0.05 % formic acid. A 40-μl aliquot of serum was injected to the LC instrument.

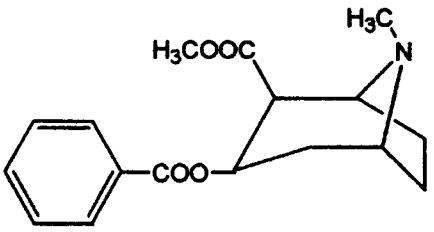
Electrospray (ES) ionization MS (/MS) was performed on a Themo Finnigan (San Jose, CA, USA) LCQ ion trap mass spectrometer with an LCQ Navigator (version 1.2) in the positive ion mode. The ES ionization conditions were: capillary temperature, 260 ℃; spray needle voltage, +5.5 kV; sheath gas flow, 100 unit; auxiliary gas flow, 30 units. The tandem MS conditions were: collision energy, 30 %; maximum injection time, 200 ms; isolation width, 2.0 Da. Full scan mode was used in both LC/MS and LC/MS/MS for mass spectral measurements and for quantification by mass chromatography.

Results

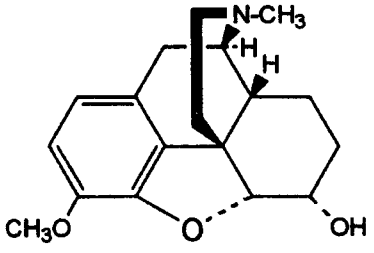
Figure 1 shows structures and molecular weights of the drugs and internal standard (IS) used in the present study. The LC/MS spectra gave $[M+H]^+$ ions constituting the base peaks at *m/z* 286,



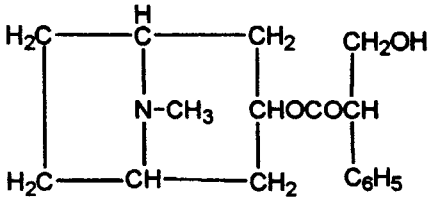
Morphine (M.W. 285)



Cocaine (M.W. 303)



Codeine (M.W. 299)



Atropine (IS; M.W. 289)

Fig. 1. Structures and molecular weights of drugs to be analyzed in the present study.

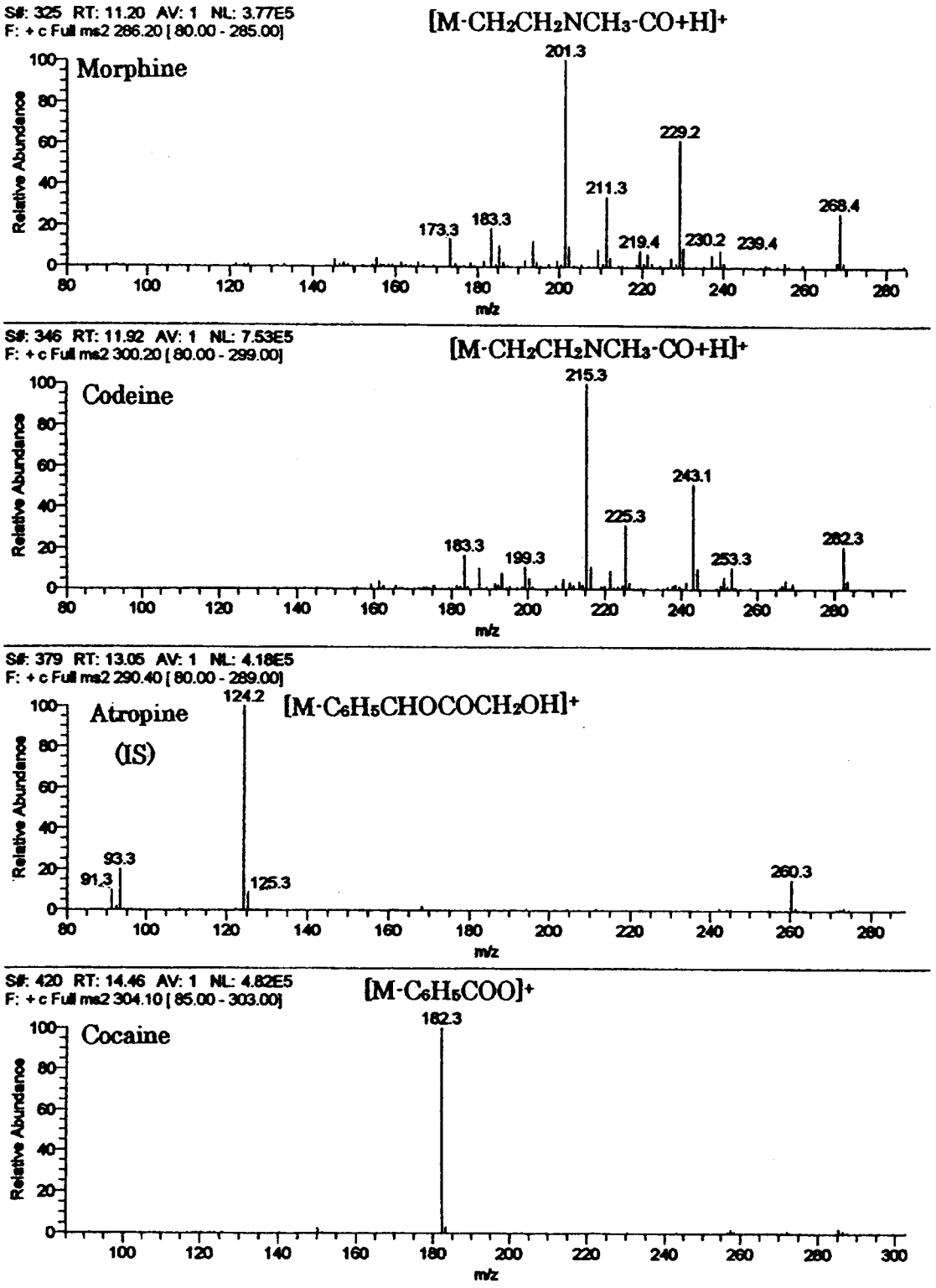


Fig. 2. Product ion mass spectra for morphine, codeine, cocaine and atropine (IS). To obtain the spectra, each protonated molecular ion obtained by LC/MS of the four compounds dissolved in solvent A was used. The concentrations of morphine, codeine, cocaine and atropine(IS) were 400, 240, 130 and 135 ng/ml, respectively.

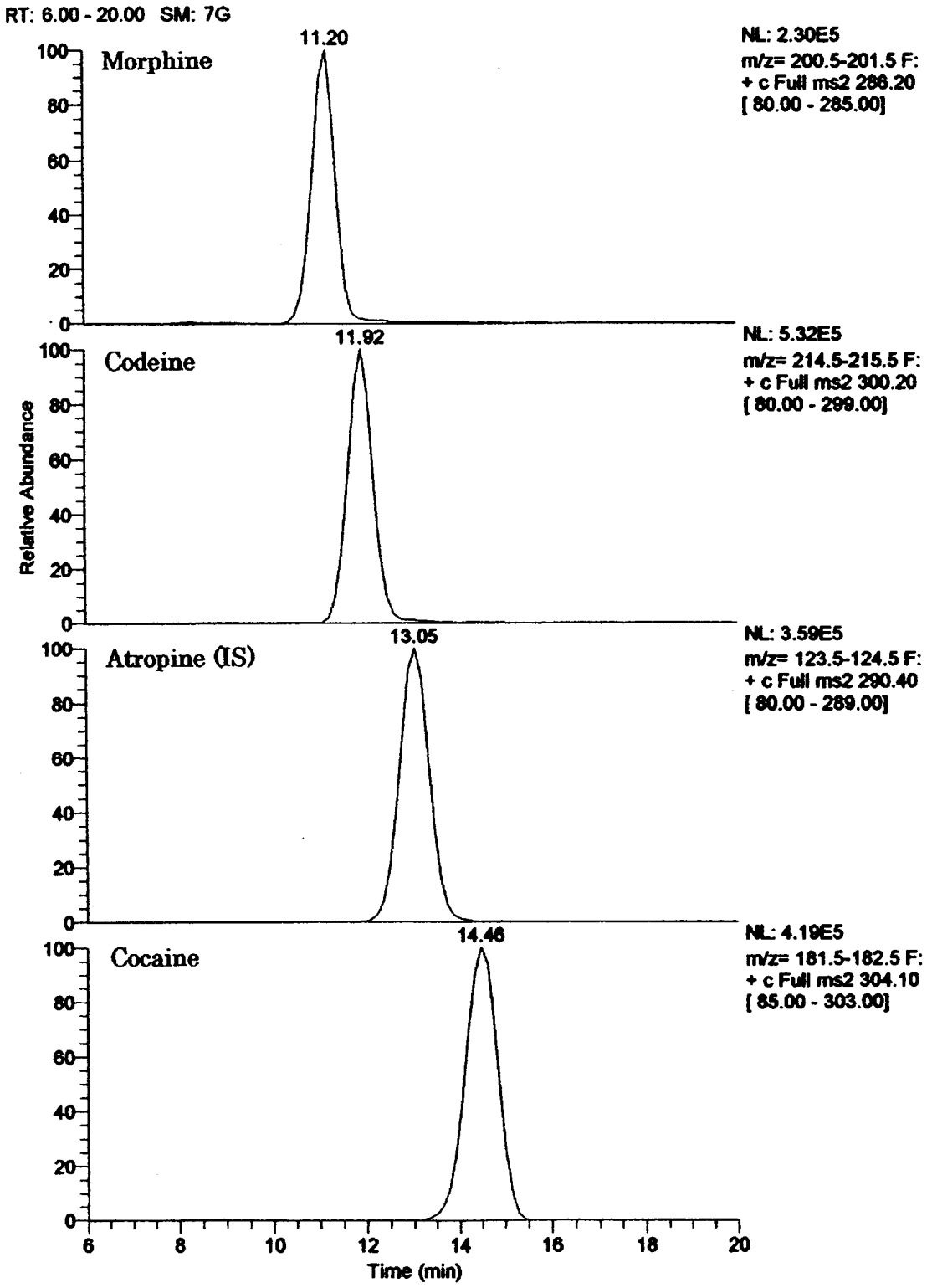


Fig. 3. Mass chromatograms of LC/MS/MS for morphine, codeine, cocaine and atropine (IS) spiked into 1 ml serum. The concentrations of the drugs were the same as specified in Fig. 2.

Table 1. Precision data for morphine, codeine and cocaine spiked into human sera

Compound	Concentration spiked (ng/ml)	Coefficient of intra-day variation (%)[a]
Morphine	100	6.98
	500	2.33
Codeine	50	3.29
	250	2.68
Cocaine	20	2.56
	100	3.66

[a] The values were obtained from 6 determinations.

300, 304 and 290 for morphine, codeine, cocaine and atropine (IS), respectively. Except the protonated molecular peaks, almost no notable peaks appeared in all LC/MS spectra.

Therefore, the protonated molecular ions were subjected to product ion formation by LC/MS/MS. The mass spectra of the product ions are shown in Fig. 2.

When the mass chromatograms obtained by LC/MS using each protonated molecular ion were compared with those obtained by LC/MS/MS using each base peak of the product ion spectra (Fig. 2), it was obvious that LC/MS/MS gave much higher sensitivity and selectivity than LC/MS. Therefore, the following experiments on quantitative analysis were made by LC/MS/MS.

Figure 3 shows mass chromatograms of LC/MS/MS for each base peak of the four compounds. The chromatograms show intense single peaks of morphine, codeine, atropine and cocaine appearing at 11.2, 12.0, 13.1 and 14.5 min, respectively.

Quantification of morphine, codeine and cocaine in human sera was made by mass chromatography of LC/MS/MS. Calibration curves were constructed for the three compounds using 135 ng/ml atropine as IS. The equations and r^2 values for the curves (y = the peak area ratio of an analyte to IS; x = the concentration of a drug in serum) were: $y = 0.003x-0.0385$ ($r^2=0.985$) in a range of 100−500 ng/ml for morphine; $y=0.0077x-0.0843$($r^2=0.9943$) in a range of 50−250 ng/ml for codeine; $y=0.0308x+0.0328$ ($r^2=0.9900$) in a range of 20−100 ng/ml for cocaine. Detection limits were 50, 20 and 10 ng/ml for morphine, codeine and cocaine, respectively.

Recovery rates from human sera were also tested; they were 50−60 % for each compound.

The precision data are shown in Table 1. The coefficients of intra-day variation were less than 7 %.

Discussion

In this report, we have used Shim-pack MAYI-ODS as an HPLC column for exclusion of large molecules such as proteins and nucleic acids together with usual chromatographic separation of small molecules. Crude biological samples, such as serum and plasma, can be directly applied to the column without any pretreatment.

The schematic illustration for the mechanisms of exclusion and separation by the Shim-pack MAYI-ODS column is shown in Fig. 4. The large molecules, such as proteins and nucleic acids, cannot pass through the porous surface of the packing material because of their size, and thus flow very fast in the column resulting in their exclusion. Although small ionized molecules can pass

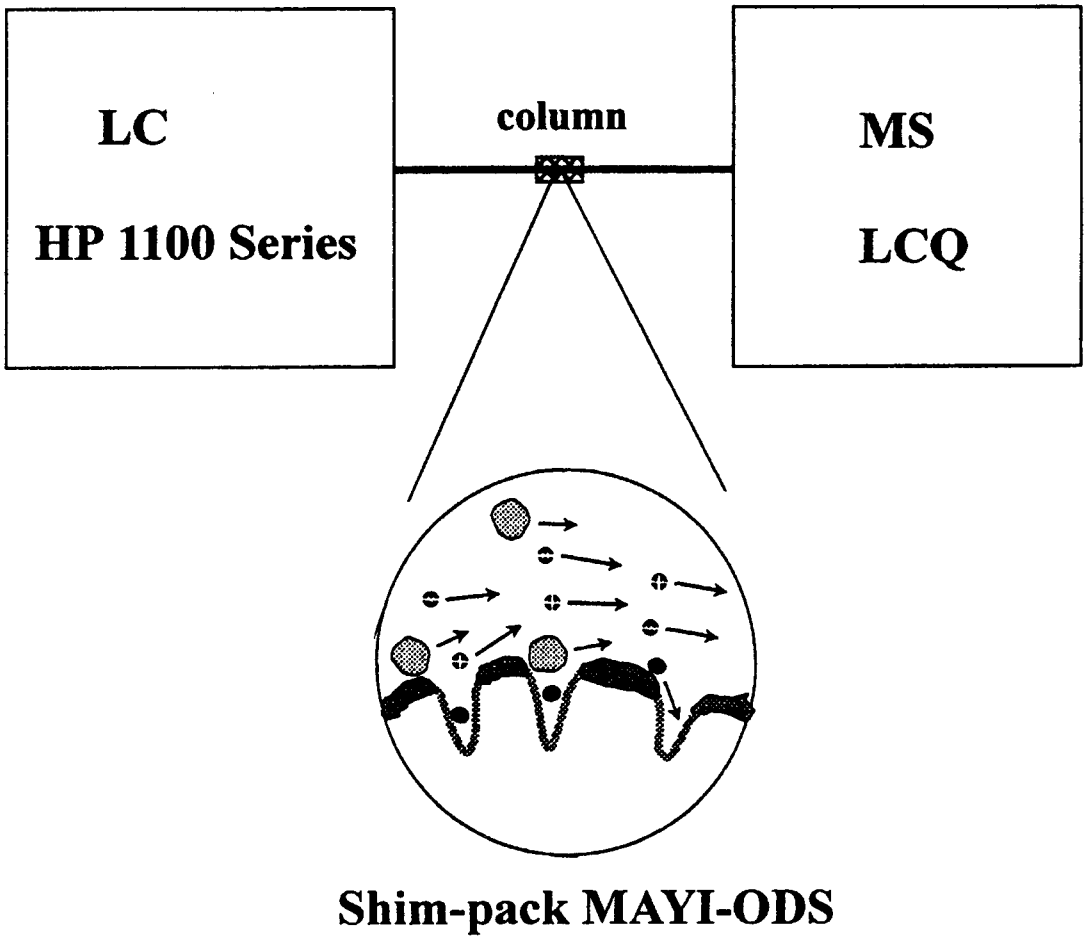


Fig. 4. Schematic illustration on separation ability of the Shim-pack MAYI-ODS column used in this study.

through the pores, but cannot be retained there and is also excluded from the column. Relatively hydrophobic small molecules are adsorbed or absorbed to the inner ODS stationary phase for chromatographic elution.

The Shim-pack MAYI-ODS packing material has been recently developed for use as a precolumn before HPLC separation [6]. The present study is the first trial for using the column for HPLC separation.

In spite of the use of LC/MS/MS, the sensitivity of the present method was not high. However, this is probably overcome by attaching a sampling loop or by increasing the volume of the packing material for larger volume injection. This line of experiments is under way in our laboratory.

In conclusion, the Shim-pack MAYI-ODS column seems very promising in analyses of drugs and toxins by HPLC, LC/MS and LC/MS/MS in extensive fields.

Acknowledgement

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