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Short Communication

Quantitative measurement of N-acetyl-L-aspartic acid in urine by gas chromatography with negative-ion chemical ionization mass spectrometry

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ABSTRACT

A highly specific and sensitive assay for N-acetyl-L-aspartic acid has been developed. The trideuterated compound was synthesized and used as an internal standard for gas chromatography with negative-ion chemical ionization mass spectrometry. Urine samples were acidified and extracted with ethyl acetate, and the compounds converted into their pentafluorobenzyl ester derivatives. Under these conditions, sub-picogram amounts of the pure derivatives could be detected. Thus, only microlitre volumes of urine samples have to be processed to achieve reliable quantification of "basal" levels of N-acetyl-L-aspartic acid.

INTRODUCTION

N-Acetyl-L-aspartic acid (NA-Asp) was discovered by Tallan *et al.* [1] and is found in high concentrations in the brains of most vertebrates. Increased levels of NA-Asp in blood, urine and cerebrospinal fluid have been found in patients with leukodystrophy [2,3]. Later, NA-Asp aciduria caused by aspartoacylase deficiency was linked with a neurological disorder resulting in spongy degeneration of the brain, Canavan desease [4], which is a form of leukodystrophy inherited as an autosomal recessive disorder [5,6].

Quantification of NA-Asp in urine is usually accomplished by gas chromatography (GC) [7], or by analysing aspartic acid after hydrolysis of NA-Asp [8].

This paper describes a highly specific and sen-

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sitive assay for NA-Asp, using GC with negative-ion chemical ionization mass spectrometry (NICI-MS) detection.

EXPERIMENTAL

Materials

Diisopropylamine, NA-Asp, aspartic acid and trideuterated acetic anhydride were purchased from Sigma (Munich, Germany). Pentafluorobenzyl bromide (PFB-Br) was obtained through Supelco (Crans, Switzerland). All other reagents or solvents were from Merck (Darmstadt, Germany).

Gas chromatography

Analysis was performed on a Carlo Erba Mega HRGC and a Finnigan 4500 mass spectrometry with as DB-5 fused-silica capillary column (J&W, Rancho Cordova, CA, USA) (15 m × 0.25 mm I.D., 0.25 μm film thickness). The column was directly connected to the ion source of the mass spectrometer. Helium was used as the carrier gas. The splitless Grob injector was kept at 290°C. The column was kept at 100°C for 1 min, and then programmed to 300°C at 20°C/min. The final temperature was held until elution was complete. After 1 min the split valves were opened to purge the injector.

Mass spectrometry

NICI spectra were recorded with methane as the moderating gas at an ion source pressure of 0.25 Torr. The electron energy and emission current were set at 120 eV and 0.25 mA, respectively. In the multiple-ion detection (MID) mode the sampling time was set at 200 ms per mass.

Pentafluorobenzyl ester formation

Pentafluorobenzyl (PFB) esters were prepared by reaction with 50 μl of PFB-Br solution in acetonitrile (7%, w/w) and 10 μl of diisopropylethylamine for 15 min at room temperature.

Preparation of trideuterated NA-Asp

A 10-mg sample of aspartic acid was dissolved in 1 ml of pyridine, and the solution was cooled on an ice-bath. After the slow addition of ice-cold deuterated acetic anhydride (200 μl), the mixture was cooled for 1 h, brought to room temperature and kept there for 3 h. The solvent and excess reagent were removed under a stream of nitrogen. The residue was dissolved in ethyl acetate and washed with 0.1 *M* hydrochloric acid. Isotopic purity was checked by GC–NICI-MS after PFB ester formation. Stock solutions were standardized against unlabeled NA-Asp and stored at −20°C.

Sample preparation

After addition of the internal standard (1 μg per 50 μl of methanol), 100 ml of urine were acidified to pH 2.0 with 0.1 *M* hydrochloric acid and extracted twice with 1 ml of ethyl acetate. The organic phase was dried under a stream of nitrogen, and the residue was derivatized as described above. After removal of excess reagent and solvent under nitrogen, the compounds were dissolved in 100 μl of ethyl acetate, and 1 μl was injected into the gas chromatograph. Quantitation was achieved by comparing the peak areas of the unlabeled compound and the internal standard. A calibration curve was constructed with known amounts of unlabeled NA-Asp.

RESULTS AND DISCUSSION

The highly electrophoric nature of pentafluorobenzyl esters, with their unique fragmentation into two resonance-stabilized structures, has already been recognized [9,10]. Thus, the PFB ester derivative of NA-Asp should show a NICI spectrum dominated by the negatively charged carboxylate anion peak at M − 181. The mass spectrum is given in Fig. 1a.

Fig. 1b shows the NICI mass spectrum of the PFB ester derivative of trideuterated NA-Asp. Since the base peak represents virtually the whole structural identity of the underivatized substance, loss of deuterium label owing to extensive fragmentation at the acetyl side-chain does not occur.

Fig. 2 shows a typical MID mass chromatogram obtained after analysis of urine sample. The

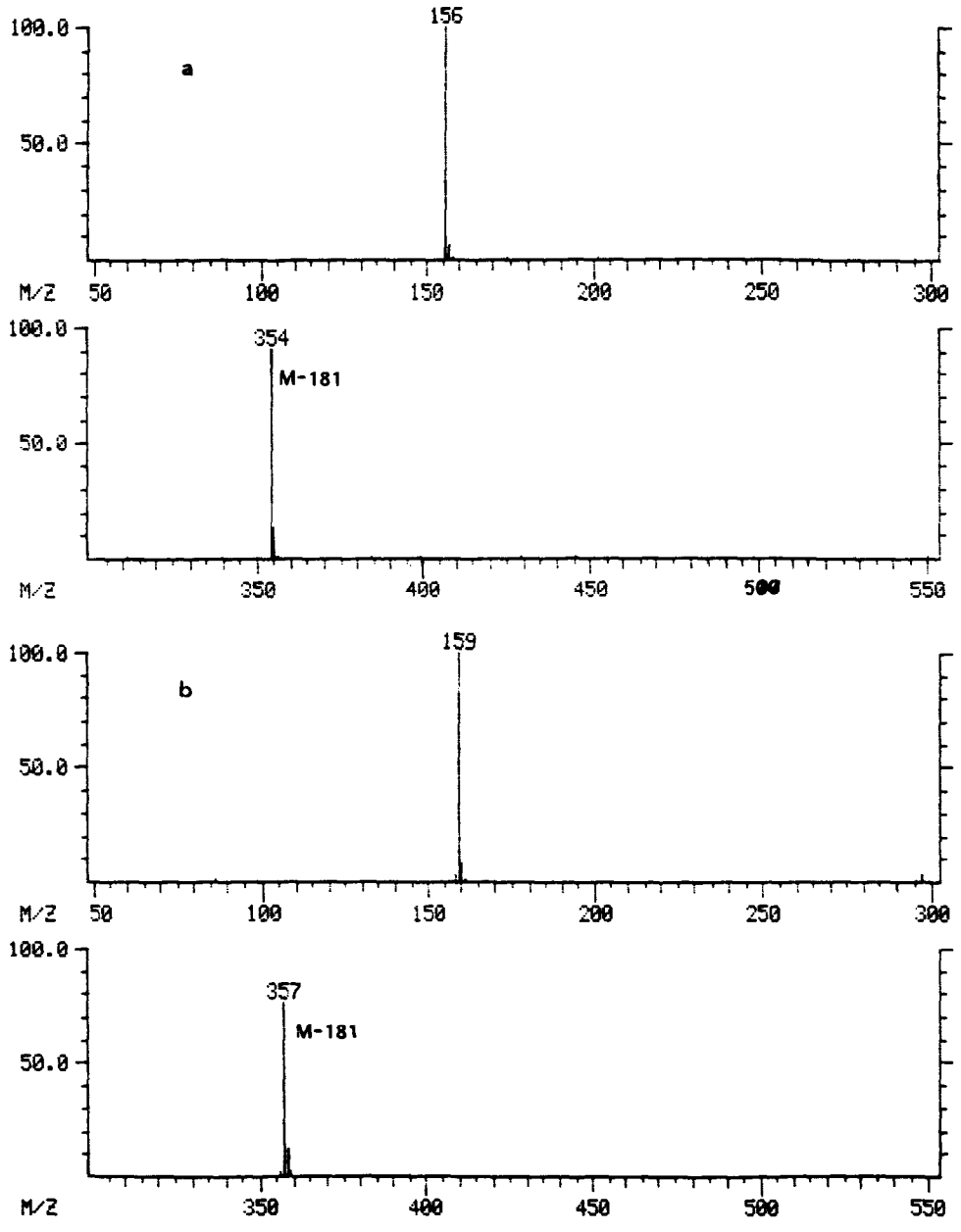

Fig. 1. NICI mass spectra of (a) unlabeled and (b) trideuterated NA-Asp PFB ester.

results of a quantitative determination of NA-Asp in the urine of four patients with expected normal values are given in Table I. The mean concentration of NA-Asp in normal subjects can thus be estimated to be 3.57 ± 0.89 n$M/\mu M$ creatinine.

The method described provides a highly sensitive and specific assay for the quantitation of NA-Asp in human body fluids. Owing to the striking response of the derivative under NICI conditions, only very small amounts of sample have to be processed, thus permitting a rapid and

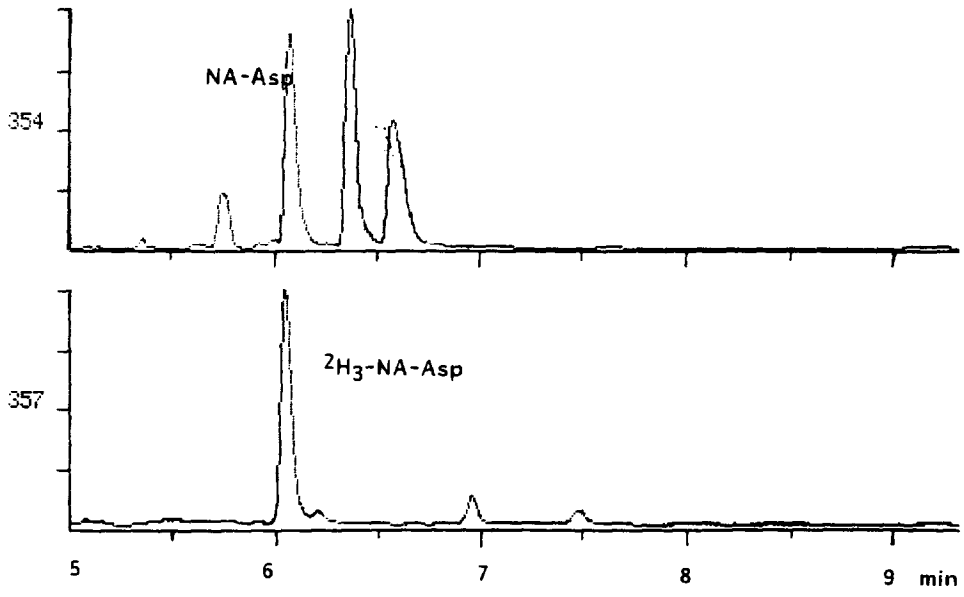

Fig. 2. Typical MID mass chromatogram obtained after GC–NICI-MS analysis of a urine sample with a normal content of NA-Asp. The compound was analysed as its PFB ester derivative.

simple work-up procedure. Solvent extracion was found to yield sufficient purity, and the low sample loading onto the GC column ensures sustained high resolution and separation efficiency.

The low limit of detection could be useful, when body fluids other than urine, *e.g.* cerebrospinal fluid, are investigated, where concentrations of NA-Asp are expected to be much lower.

TABLE I

CONCENTRATIONS OF NA-Asp IN URINE OF FOUR NORMAL SUBJECTS

Patient	Creatinine (μmol/ml)	NA-Asp (nmol/μmol of creatinine)
1	6.11	4.3
2	14.60	3.2
3	6.46	2.3
4	9.20	4.5
Mean		3.57 ± 0.89

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REFERENCES

1 H. H. Tallan, S. Moore and W. H. Stein, *J. Biol. Chem.*, 219 (1956) 257.
2 E. A. Kvittingen, G. Guldal, S. Borsting, I. O. Skalpe, O. Stokke and E. Jellum, *Clin. Chim. Acta*, 158 (1986) 217.
3 L. Hagenfeldt, I. Bollgren and N. Venizelos, *J. Inher. Metab. Dis.*, 10 (1987) 135.
4 R. Matalon, K. Michals, D. Sebesta, M. Deanching, P. Gashkoff and J. Casanova, *Am. J. Med. Gen.*, 29 (1988) 463.
5 L. van Bogaert and I. Bertrand, in *Spongy Degeneration of Brain in Infancy*, North Holland Publishing, Amsterdam, 1967, pp. 3–132.
6 D. S. Buchanan and R. L. Davis, *Neurology*, 15 (1965) 207.
7 M. Miyake, Y. Kakimoto and M. Sorimachi, *J. Neurochem.*, 36 (1981) 804.
8 S. I. Goodman and S. P. Markey, in *Diagnosis of Organic Acidemias by Gas Chromatography–Mass Spectrometry*, Alan R. Liss, York, 1981, pp. 3–24.
9 H. J. Leis, M. Leis, W. Welz and E. Malle, *J. Chromatogr.*, 529 (1990) 299.
10 H. J. Leis, E. Malle, R. Moser, J. Nimpf, G. M. Kostner, H. Esterbauer and H. Gleispach, *Biomed. Environ. Mass Spectrom.*, 13 (1986) 483.