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Letter to the Editor

Determination of pyridostigmine plasma concentrations by high-performance liquid chromatography

Sir,

Several methods have been reported for the determination of pyridostigmine plasma concentrations, including spectrophotometry [1], enzymic determination [2], radioimmunoassay [3] and gas–liquid chromatography [4–7] as well as sensitive and specific high-performance liquid chromatographic (HPLC) assays [8–13]. Easy and rapid pyridostigmine plasma extraction procedures using Sep-Pak C_{18} cartridges are described by Ellin *et al.* [12], with a detection limit of 40 ng/ml by subsequent HPLC separation using an analytical C_{18} column. Lower limits of detection (10 ng/ml) can be obtained if cation-exchange extraction is combined with HPLC separation using an analytical cyano column [13].

In our experience, a limit of detection of *ca.* 1–2 ng/ml (at a signal-to-noise ratio of 2) can be obtained when slightly modified extraction procedures according to Ellin *et al.* [12] are combined with the HPLC separation reported by Matsunaga *et al.* [13].

EXPERIMENTAL

ng/ml by assaying plasma samples spiked with known pyridostigmine concentrations. The intra- and inter-day precisions of the assay were estimated by measuring plasma standards with 10 and 50 ng/ml pyridostigmine at the first day (n = 10) and on the nine following days.

A 2-ml volume of plasma was diluted with 4 ml of 0.5 M phosphate buffer (pH 10.6) and passed through an activated (5 ml of methanol, 5 ml of water) Sep-Pak C_{18} cartridge (Waters, Eschborn, F.R.G.). Afterwards, the cartridge was purged with 5 ml of 0.05 M phosphate buffer (pH 10.6) and 5 ml of methanol. Pyridostigmine was then eluted with 3 ml of 1% acetic acid in methanol and, after evaporation to dryness (nitrogen, 60°C), the residue was redissolved with 60 μl of water. Aliquots of 50 μl were injected into the HPLC column

RESULTS AND CONCLUSIONS

Fig. 1 shows chromatograms of a blank plasma sample (A) and of a plasma sample withdrawn from a patient who had 2 h previously received 60 mg of pyridostigmine (B). The drug concentration calculated from this chromatogram was 16 ng/ml The calibration curve was linear in the range studied, and has a slope of 0.941 and an y-intercept of -0.0745; r = 0.999 [y = pyridostigmine plasma concentration (ng/ml), and x = peak height (mm)]. The lower limit of detection was *ca.* 1–2 ng/ml at a signal-to-noise ratio of 2.

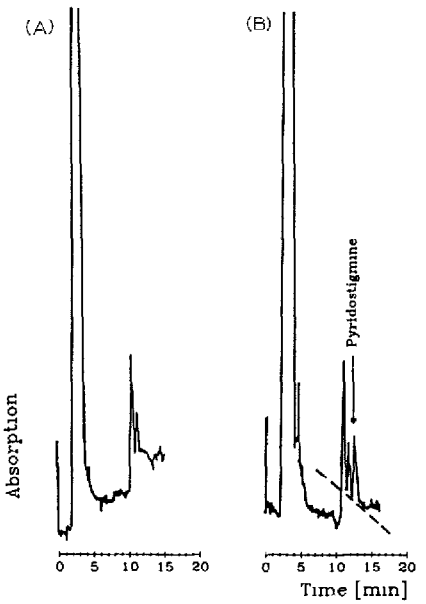

Fig 1. Chromatograms of (A) a blank plasma sample and (B) a plasma sample withdrawn from a patient 2 h after administration of 60 mg of pyridostigmine The drug concentration calculated from this chromatogram was 16 ng/ml. The pyridostigmine peak height was determined by the tangent skimming technique

TABLE I

ACCURACY OF THE ASSAY

Concentration of pyridostigmine (ng/ml)		Accuracy[a] (%)
Spiked	Found	
5	5	0 0
10	10	0 0
20	19	5 0
50	48	4 0
100	99	1.0
200	204	2.0
Mean ± S D.		2.0 ± 2 1

[a] Calculated according to ref. 14

The accuracy of the method is shown in Table I: the theoretical concentrations (spiked concentration) agreed well with the assayed concentrations (found concentration), with an accuracy of 2 ± 2.1% (mean ± S.D.).

For intra- and inter-day precision studies, the mean concentration, S.D. and coefficient of variation (C.V.) are shown in Table II.

This method combines the extraction procedures of Ellin *et al.* [12] with the HPLC separation reported by Matsunaga *et al.* [13]. The lower limit of detection is *ca.* 10–40-fold below that of both assays, and in clinical routine the method allowed reliable and reproducible determination of pyridostigmine plasma con-

TABLE II

INTRA- AND INTER-DAY PRECISION FOR PYRIDOSTIGMINE

	Spiked concentration (ng/ml)	Found concentration (mean ± S D , $n = 10$) (ng/ml)	C.V. (%)
Intra-day	10	9 9 ± 1 1	11 1
	50	50 0 ± 2 2	4 4
Inter-day	10	10.5 ± 1.0	9.5
	50	49 8 ± 2.3	4.6

centrations in more than fifty myasthenia gravis patients, also in a low concentration range of 2–10 ng/ml.

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