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Note

Determination of calcium pantothenate in multivitamin preparations by high-performance liquid chromatography

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Several methods have been described for determination of calcium pantothenate, using thin-layer chromatography[1], gas chromatography[2], colorimetry[3–5], fluorimetry[6] and liquid chromatography[7]. The method described in this paper is low cost, accurate and sensitive enough to determine concentrations such as 1 mg/100 ml. It was developed for the rapid detection of calcium pantothenate in multivitamin tablets during pharmaceutical quality control analysis.

EXPERIMENTAL

Reagents and materials

Acetic acid was purchased from Fluka and was puriss grade. Water was filtered through a Millipore device and its resistivity was at least 18 mΩ. It was filtered before use through a 0.22-μm inert filter (Sartorius, Ref. SM 11607).

Apparatus

The analaysis was performed on a liquid chromatograph equipped with a Model 6000 A pump (Waters, U.S.A.) and connected to a R 400 differential refractometer (Waters). Injection was through a septumless injector with a 100-μl sample loop. The electronic unit was connected to a reporting integrator (Hewlett-Packard Model 3390 A).

Preparation of eluent

Glacial acetic acid (50 ml) was added to 950 ml of 18 mΩ water. The solution was stirred magnetically for 60 sec and then degassed by helium (15 min), before being filtered through a 0.22-μm filter.

Calcium pantothenate standard preparation

Calcium pantothenate (100 mg) was weighed accurately into a 100-ml volumetric flask. Demineralized water (40 ml) was added and the flask was shaken until the compound dissolved. The sample was taken to volume with elution solvent. The solution was filtered through a 0.22-μm filter. Dilutions were made with this stock solution to obtain solutions containing 0.5, 0.25, 0.1 and 0.05 mg/ml.

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Sample preparation

One tablet was put in a 50-ml volumetric flask, 40 ml of eluent were added and the flask was placed in an ultrasonic bath until the compound dissolved. The sample was taken to volume with eluent, and the solution filtered through a 0.22-μm inert filter. Samples were introduced manually in 100-μl volumes.

Chromatographic conditions

The analysis was performed on Nucleosil 7 C_{18} reversed phase (Macherey Nagel) in a 20-cm long, Perkin-Elmer HS 5 C_{18} stainless-steel column. The flow-rate was 2 ml/min and the sensitivity of the RI detector was 8 (the range is from 1 to 128 full scale). Special attention was given to baseline drift owing to effect of temperature, and the detector optical unit was thermostatted at 35°C. Under these conditions, the retention time of calcium pantothenate was 4.50 min and the separation was good enough for its determination in samples (Fig. 1). All chromatograms were obtained isocratically at room temperature.

RESULTS AND DISCUSSION

Linearity

Calcium pantothenate showed a linear response from 20 to 400% of the normal injection concentration (Table I). The linear correlation coefficient was found to be more than 0.99869 and the intercept was less than 2.1% of the response of the normal injection concentration. This single-point standardisation was deemed to be adequate.

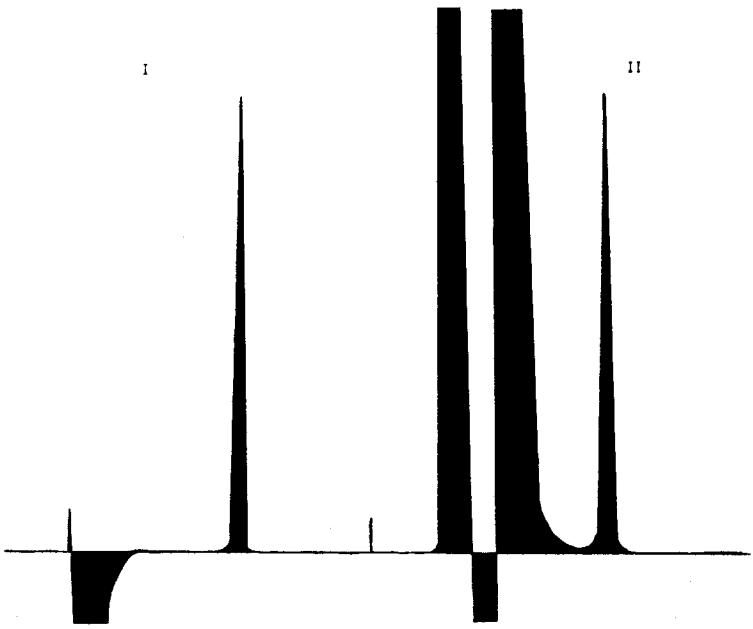

Fig. 1. Typical chromatogram obtained by injection of a standard solution (I) and of a sample solution (II).

TABLE I

LINEARITY STUDY

Concentration of soln. (mg %)	*Amount injected (μg)*	*Area of peak detected*	*Mean area of peak*	*Standard deviation*	*Relative standard deviation*
5	5	437,890 445,590 433,230 435,520 417,800 415,270 435,400 451,000	433,962.5	12,285.6	0.028
10	10	756,310 757,150 725,340 731,180 749,410 735,170 725,910 740,540	740,126.2	12,891.9	0.017
25	25	1,824,000 1,799,600 1,832,800 1,832,900 1,833,400 1,820,200 1,790,000 1,813,880	1,818,472.5	16,425.5	0.009
50	50	3,682,100 3,683,900 3,676,400 3,672,000 3,680,000 3,670,300 3,672,000 3,685,900	3,677,820	5995.6	0.001
100	100	7,973,200 7,961,100 7,915,700 7,998,400 8,060,500 8,113,700 8,189,100 7,937,800	8,031,187.5	94,426.6	0.011

Chromatographic precision

The chromatographic precision was determined by making eight injections from a single freshly prepared solution of calcium pantothenate in water on each of

TABLE II

DAY-TO-DAY REPRODUCIBILITY OF INJECTION OF REPLICATE 500 ng/ml SAMPLES OF CALCIUM PANTOTHENATE

Injection No.	*Day 1*	*Day 2*	*Day 3*
1	3,682,100	3,684,100	3,687,000
2	3,683,900	3,679,200	3,674,100
3	3,676,400	3,628,000	3,693,000
4	3,672,000	3,637,000	3,642,000
5	3,680,000	3,645,000	3,653,200
6	3,670,300	3,682,000	3,668,000
7	3,672,000	3,685,400	3,693,200
8	3,685,900	3,662,300	3,634,000
Mean value of area	3,677,825	3,662,875	3,644,187.5
Standard deviation	5995.6	23,286.3	74,298.6
Relative standard deviation	0.0016	0.0063	0.0203

TABLE III

ACCURACY AND ASSAY PRECISION

Sample	*Area of peak detected*	*Mean area of peak*	*Calcium weight (mg)*	*Actual weight (mg)*	*Recovery (%)**
1 (Standard)	284,889 270,003 267,404 273,152 267,379	272,565	25	25	–
2	269,427 270,963 274,188 269,081 268,582	270,448	24.80	24.80	100
3	264,320 263,168 275,904 275,430 277,516	271,267	24.88	24.90	99.9
4	272,064 274,956 276,224 276,481 277,158	275,176	25.23	25.30	99.7

* Average is 99.86% with a relative standard deviation of ±0.15%.

TABLE IV

ASSAY OF COMMERCIALLY AVAILABLE CALCIUM PANTOTHENATE TABLETS

Batch No.	*Concentration as a percentage of theoretical concentration*
1	98.4
2	104.4
3	100
4	100
5	96.8
6	102.0
7	98.7
8	98.8

three days. The precision was calculated each day as the relative standard deviation. The chromatographic precision was taken to be the average of the three relative standard deviations and was found to be 0.94% (Table II).

Accuracy and assay precision

An estimate of the procedural accuracy was obtained by assaying three replicate weighings of calcium pantothenate *versus* a fourth weighing used as a standard. The reproducibility of the recoveries was used as an estimate of assay precision (Table III).

Detection limit

The detection limit of the product used for dosage was considered to be the amount injected that gave a peak area equal to three times the mean value of "baseline noise", the detector and integrator being set at maximum sensitivity. The test showed that detection limit was 50 ng injected (*i.e.* 100 μl of a solution at 500 ng/ml).

Application to commercial dosage forms tested

Several batches of tablet dosage forms containing vitamins (thiamine, pyridoxine, nicotinamide, riboflavine) formulated in this laboratory were determined using this method (Table IV). All laboratory samples were within their shelflife expiration date. When the products were spiked with calcium pantothenate, the assay detected the appropriate amounts, showing that there was no interference with other vitamins.

CONCLUSION

A method of analysing calcium pantothenate in vitamin mixtures is described which allows the quantitative determination of this compound in less than 5 min. This is to be compared with the less specific[5] and somewhat time consuming methods[2] described in previous papers.

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