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Assay Methods for a new Analgesic Enkephalin Analogue CDRI compound No. 82/205

A. K. DWIVEDI, M. KHANNA AND S. SINGH
Divn. of Pharmaceutics, C.D.R.I., Lucknow - 226001

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HPLC and TLC-densitometric methods for the estimation of L-tyrosyl-D-alanyl-glycyl-L-N-methyl phenyl alanyl glycyl-N-isopropyl amide [compound 82/205] in bulk samples and formulations are described. The calibration curves were linear in the range of 4-40 µg/ml for HPLC and in the range of 0.5 - 20 µg for TLC-densitometric method.

STANDARD compound 82/205¹⁻⁵ is a pale yellow powder with molecular weight 569. It was obtained from this institute. Methanol and chloroform used were of AR grade. Dual wavelength tic-scanner (Shimadzu model CS-910) fitted with Shimadzu U-235 data recorder, precoated silicagel plates 60F254 with a layer thickness 0.25 mm [E. Merck] and micro syringe (50 µl, Top) were used for TLC densitometric analysis. The hplc system consists of a Perkin Elmer 250 solvent delivery pump,

Perkin-Elmer LC 235 diode array detector, Rheodyne 7125 injector fitted with a 20 µl loop, a C18 column Lichrospher 100 RP-18, 5 µm, 250 x 4 mm (E. Merck) and GP 100 printer plotter (Perkin Elmer).

Five mg of 82/205 was dissolved in 10 ml methanol to get a standard solution with concentration of 0.5 ug/ul. Stability of 82/205 in this solution was also checked. It was observed that not more than 5% of 82/205 decomposed when kept in its solution form for 24 h, at room temperature.

Bulk drug sample or formulation equivalent to 5 mg of

Table - 1 : Inter and intra assay variations

HPLC method

Intra assay variation				inter assay variation		
Conc. Inj. (µg/ml)	Conc. Found (Mean ± S.D.) (µg/ml)	% C. V.	% DFA	Conc. Found (Mean ± S. D.) (µg/ml)	% C. V.	% DFA
4.0	4.00±0.14	3.38	-0.05	4.01±0.32	8.09	0.13
10.0	10.72±0.17	1.63	7.23	10.51±0.14	1.33	5.05
20.0	10.40±0.24	1.26	-2.61	19.67±0.54	2.73	-1.66
30.0	30.26±0.17	0.58	0.86	30.08±0.34	1.12	0.27
40.0	40.81±0.05	0.11	2.03	40.50±0.27	0.67	1.24

TLC densitometric method

Inter assay variation				Intra assay variation		
Conc. Loaded (µg/ml)	Conc. Found (Mean ± S.D.) (µg/ml)	% C. V.	% DFA	Conc. Found (Mean ± S. D.) (µg/ml)	% C. V.	% DFA
0.5	0.473±0.026	5.31	-5.30	0.473±0.015	3.30	-5.40
4.0	4.160±0.116	2.79	4.00	4.033±0.061	1.52	0.83
10.0	10.220±0.081	0.79	2.17	10.073±0.175	1.73	0.72
14.0	13.970±0.220	1.57	-0.19	13.973±0.165	1.18	-0.19
20.0	20.020±0.172	0.86	0.10	20.023±0.329	1.64	0.12

compound was extracted with methanol (3 x 3 ml), volume was made to 10 ml and filtered.

Different amounts (0.5 to 20 µg) of 82/205, 20 µl of blank methanol and sample solution equivalent to 10 µg were loaded on tlc plate. Chromatography was carried out in a glass tlc tank saturated with the upper layer of butanol : acetic acid : water (4 : 1 : 5) or with 30 % methanol in chloroform. Plates were run at least to the height of 15 cm. Plates were removed, air dried and spots were developed by spraying through ninhydrin solution then heating at 100° 5 minutes. Pink purple spots [Rf - 0.66 (BuOH : H₂O ; AcOH)] and [Rf-0.35 (CHCl₃ : MeOH)] developed were scanned at 530 nm using single wavelength reflection mode with background subtraction and a light beam of 1 x 10 mm and chart speed 40 mm/min. Colour intensity of the spots were recorded in the chromatograms. Peak areas

were measured and plotted against concentrations to get a calibration curve. The concentrations of 82/205 was determined by using standard curves. Recoveries were calculated by adding known concentration of 82/205 to preanalysed samples.

The mobile phase for hplc consisted of a mixture of methanol, water and tri-fluoro acetic acid (55 : 45 : 0.015). The compound eluted out at 5.8 minute using a flow rate of 1.0 ml per minute and a detection wave-length of 275 nm.

In tlc-densitometry the calibration curve is linear in the range of 0.5 - 20 µg with R = 0.9989. The reproducibility and accuracy of the method was checked by inter/ intra assay variations and % deviation from actual concentration respectively, which were found to be less than 5%. Results

indicate high accuracy and precision of the method. In HPLC the calibration curve is linear in the range 4 - 40µg/ml, with correlation coefficient $R = 0.9996$. The reproducibility of the method was checked by inter and intra assay variations and the accuracy of the method was checked by % deviation from actual concentration, both were found to be less than 5%. The content of the compound was calculated from the average peak height of six replicates by using the formula

$$\text{Unknown conc.} = K \times \text{Peak height} + B,$$

Where $K = 2.7276$ and $B = 0.7111$ (for tlc-densitometry)

and $K = 6.6940$ and $B = -0.3305$ (for hplc method)

The present methods provided sensitive assay methods with proper resolution of 82/205. No interference from the other constituents of formulations were observed. Table I shows the inter and intra assay variations results.

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Performance evaluation of Tamarind seed polyose as a binder and in sustained release formulations of low drug loading

D. KULKARNI, A. K. DWIVEDI AND S. SINGH

Divn. of Pharmaceutics, Central Drug Research Institute, Lucknow - 226001

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Evaluation of tamarind seed polyose as a binder for tablet dosage forms was taken up for the wet granulation as well as direct compression methods. The drug release sustaining properties of tamarind seed polyose polymer were also studied using 5 mg of terbutaline sulphate matrices. The results indicated that tamarind seed polyose could be used as binder for wet granulation and direct compression tableting methods as well as a suitable polymer for sustained release formulations of low drug loading.

THE extraction process for Tamarind Seed Polyose (TSP), a natural polymer^{1,2} obtained from the seeds of *Tamarindus Indica* has been developed at this Institute. An assessment of its bindings properties in wet granulation method and as a dry binder in tablet dosage forms was undertaken vis-a-vis the binding properties of

binders such as starch, gelatin, methyl cellulose, sodium carboxymethyl cellulose and polyvinyl pyrrolidone. Tablets prepared were evaluated for uniformity of weight, hardness, friability³, and disintegration tests⁴. A recent study showed slow drug release for tablets compressed form TSP with high drug loading of verapamil hydrochloride^{5,6}. The present study also reports the use of TSP as a polysaccharide polymer in the design of solid controlled release dosage

*For correspondence