

A high performance liquid chromatographic method for the estimation of diethylcarbamazine content in medicated salt samples

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Abstract

A simple and reproducible method for the estimation of diethylcarbamazine citrate (DEC) by high performance liquid chromatography (HPLC) in DEC-medicated salt was developed. HPLC analysis was conducted with a mobile phase containing acetonitrile/phosphate buffer (20 mM KH_2PO_4 , adjusted to pH 3.2 with 10% *ortho*-phosphoric acid) in the ratio of 1:9 and at a flow rate of 1.5 ml/min. A Phenomenex C8 column (15 cm \times 4.6 mm) of 5 μm particle size was used for the analysis. Analysis was done at UV 210 nm, 0.02 a.u.f. and 40 °C. The coefficient of variation was <10% in the range of 1–25 $\mu\text{g/ml}$ and the minimum detectable level was 0.5 $\mu\text{g/ml}$. The quality of DEC-medicated salt prepared by two methods was analyzed by using the HPLC method. In spray drying method, 29 and 71% of the samples and in rotating drum method, 9 and 12% of samples were found to contain DEC at 0.15–0.25% and >0.25%, respectively. Thus, this quick and simple HPLC method for the estimation of DEC could play a vital role in checking the quality of the DEC medicated salt used for the control of filariasis. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: HPLC; Diethylcarbamazine; Medicated salt; Filariasis

1. Introduction

Lymphatic filariasis is caused by the parasitic nematodes *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* transmitted by mosquito vectors. Over one-third of the World's population at risk for lymphatic filariasis lives in India (WHO, 1992). It is considered as a major obstacle to

economic development in endemic countries and has been identified as the second leading cause of permanent and long-term disability (WHO, 1994). Control options for lymphatic filariasis are diverse and range from chemotherapy to vector control by the application of insecticides, polystyrene beads and biocides (WHO, 1992, 1994; Ottesen and Ramachandran, 1995).

DEC applied as an additive to common table/cooking salt is effective as a control agent and may have the advantage of easy delivery and reduced costs (WHO, 1994; Ottesen and Ra-

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machandran, 1995). Diethylcarbamazine (DEC) has been used for many years to treat human lymphatic filariasis. DEC is extremely stable, and is not affected by autoclaving or by cooking (Gelband, 1994). For a successful mass chemotherapy programme an effective drug delivery is essential. In the case of the medicated salt programme the success depends on both the delivery mechanism and the DEC content of the medicated salt. For the mixing of the salt with DEC, different methods such as spray drying (Fan, 1990) and rotating drum (Reddy and Venkateswaralu, 1996) are commonly employed. Under such situations the estimation of the DEC content is a pre-requisite for the proper implementation of the chemotherapy programme.

Presently, for estimation of DEC, spectrophotometric (Lubran, 1950; Rao and Subramanyam, 1970; Ramachandran, 1973), gas chromatographic (Bogan, 1977; Allen et al., 1979; Ptaff et al., 1978; Lee et al., 1997), ELISA (Mitsui et al., 1996; Mitsui and Aoki, 1998) and NMR methods (Jaroszewski et al., 1996) are mainly used for the analysis in biological fluids. All these methods except the ELISA and NMR methods require time-consuming extraction and are unsuitable for larger sample size. The utility of High Performance Liquid Chromatographic (HPLC) method suitable for the analysis of DEC content in medicated salt samples is highlighted in this communication.

2. Materials and methods

Pure Diethylcarbamazine citrate used for the standardization was obtained from Sigma Chemical Co, St Louis, MO, USA. HPLC grade acetonitrile, potassium dihydrogen phosphate and ortho phosphoric acid of Analar grade were procured from M/s S.D.Fine Chem Ltd, Boisar, India. Cellulose nitrate membrane filter was procured from Toyo Roshi Kaisha Ltd, Japan.

An LC-6A Shimadzu HPLC equipped with dual pump, DGU-6A online degasser, CTO-6A column oven, SPD-6AV UV detector, C-R6A Chromatopac, SCL-6A system controller and a Rheodyne injector were used for the analysis.

2.1. Standardization of DEC by HPLC

Injection volume was kept as 10 μ l. A standard stock solution of DEC citrate was made by dissolving 10 mg of DEC-citrate in 10 ml of triply distilled water. Five standard solutions of 1 ml each were made from the stock solution in triply distilled water so as to get corresponding peak areas for 0.025, 0.05, 0.1, 0.2 and 0.5 μ g of DEC by the injection of 10 μ l of each standard solution into HPLC. The corresponding peak areas obtained were used to get a linear regression equation. This equation was used for the quantitative estimation of DEC in medicated salt samples. The HPLC assay precision was tested at five different DEC levels over a range of 0.5–25 μ g/ml in aqueous and in 1% saline samples.

2.2. HPLC analytical conditions

HPLC analysis was conducted under the following conditions: mobile phase—acetonitrile/phosphate buffer (20 mM KH_2PO_4 , adjusted to pH 3.2 with 10% orthophosphoric acid) in the ratio of 1:9 and at a flow rate of 1.5 ml/min. A Phenomenex C8 column (15 cm \times 4.6 mm) of 5 μ m particle size was used for the analysis. Analysis was done at UV 210 nm, 0.02 a.u.f. and 40 °C.

2.3. Sample preparation and HPLC analysis

DEC-Medicated salt samples (196) that were prepared by spray drying or rotating drum method, were received from the study areas of Tamil Nadu and Kerala where pilot DEC-medicated salt programme was launched for the control of Bancroftian and Brugian filariasis. The techniques (Gelband, 1994; Fan, 1990; Reddy and Venkateswaralu, 1996) of preparing DEC-medicated salt involve either adding crushed DEC tablets in a hand turned or electric mixing drum (rotating drum method) or spraying a DEC solution on to salt and then drying it (Spray drying method). The samples were collected randomly from the houses and kept in polyethylene bags for the DEC content analysis.

Samples for HPLC analysis were prepared by accurately weighing 1gm of the medicated salt

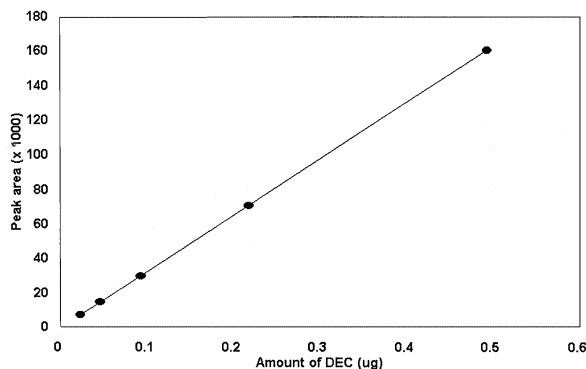


Fig. 1. Calibration curve for the estimation of DEC by HPLC.

sample and dissolving it in 10 ml of triply distilled water. Dilutions (1:9) were made whenever required with triply distilled water. All the samples were filtered through 0.2 μm cellulose nitrate filter before injecting to the HPLC. Samples were prepared in duplicate for each coded batch of the medicated salt.

3. Results

3.1. Standardization of DEC by HPLC

A calibration curve (Fig. 1) was constructed by plotting the peak area in the Y-axis and the concentration in the X-axis. The unknown concentrations were obtained by solving the linear regression equation for the calibration curve $Y = 3.2 \times 10^5 X - 4.2 \times 10^2$ for X. The calibration

curve showed good linearity with a correlation coefficient of 0.9985.

Typical chromatograms of aqueous and saline samples of DEC (10 $\mu\text{g}/\text{ml}$) are shown in Fig. 2a and b, respectively. The peak corresponding to 3.3 min has been due to DEC. The HPLC assay precision was tested at five different DEC levels over a range of 0.5–25 $\mu\text{g}/\text{ml}$ in aqueous and in 1% saline samples and the results are presented in Table 1. The estimated DEC content in all the samples were near to the exact DEC added. The coefficient of variation was < 10% in the range of 1–25 $\mu\text{g}/\text{ml}$ and the minimum detectable level was 0.5 $\mu\text{g}/\text{ml}$.

3.2. DEC content in medicated salt samples

A total of 196 DEC-medicated salt samples—134 from rotating drum method and 62 from spray drying method were analyzed for DEC content. Based on the results, the samples were grouped into four categories as given in Table 2. When the level of DEC was between 0.0 and 0.005% it was classified under ‘very low’. Similarly for ‘low’ for 0.005–0.15%, ‘optimum’ for 0.15–0.25% and ‘high’ when the DEC content was more than 0.25%. In spray drying method 29 and 71% of the samples were falling under ‘optimum’ and ‘high’ category whereas in the case of rotary drum method 43.3 and 35.8% of the samples were under ‘very low’ and ‘low’ category, respectively, and only 9 and 12% of the samples were in ‘optimum’ and ‘high’ category.

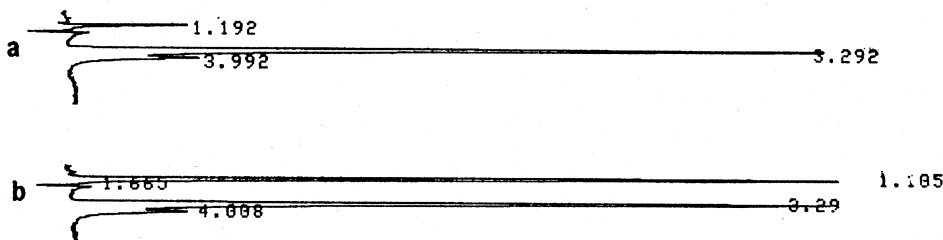


Fig. 2. Chromatograms for DEC (10 $\mu\text{g}/\text{ml}$) in aqueous (a) and saline (b) solutions.

Table 1
Precision of DEC estimation by HPLC

Sample	DEC added (µg/ml)	DEC estimated (µg/ml ± S.D.)	% Recovery	Coefficient of variation	Number of assays
Aqueous	25	22.7 ± 0.7	90.9	3.2	5
	10	9.6 ± 0.2	96.0	1.7	5
	5	4.5 ± 0.1	89.9	1.1	5
	1	0.9 ± 0.04	94.1	3.9	5
	0.5	0.4 ± 0.03	88.5	7.7	5
Saline (1%)	25	26.5 ± 0.4	105.8	1.7	5
	10	9.7 ± 0.5	96.7	5.3	5
	5	5.5 ± 0.2	109.4	4.5	5
	1	0.9 ± 0.02	93.8	1.7	5
	0.5	0.5 ± 0.02	95.2	5.1	5

4. Discussion

Diethylcarbamazine-medicated salt is a safe and effective control measure for the control of lymphatic filariasis. With good population coverage, it can stop transmission and control clinical disease in endemic areas (Gelband, 1994). The success of DEC medicated salt programme depends on both the delivery mechanism and the DEC content of the medicated salt.

Most of the reports on the estimation of DEC involve the cumbersome and time consuming extraction procedures involving the alkaline hydrolysis followed by organic solvent extraction and are mainly aimed at the metabolite studies in biological fluids. The spectrophotometric methods for estimating DEC (Lubran, 1950; Rao and Subramanyam, 1970; Ramachandran, 1973) based on the formation of ion-pairs lack specificity and sensitivity. The initial gas chromatographic method for measuring DEC in animal plasma and tissues (Bogan, 1977) lacks sensitivity. The modified gas chromatographic method (Allen et al., 1979) improved the sensitivity and Lee et al. (1997) applied a solid phase extraction technique before GC analysis of DEC metabolites in biological samples. Though solvent extraction is not required in ELISA method, it involves the use of specific anti-DEC antibody (Mitsui et al., 1996) and in NMR method (Jaroszewski et al., 1996)

sample has to be mixed with 10% of deuterium oxide as a spectrometer field frequency lock.

In the present HPLC method, no organic solvent extraction or hydrolysis of the sample is required. The medicated salt samples have to be dissolved in triply distilled water and filtered through a membrane filter and has to be injected to HPLC. Since the mobile phase contains only 10% HPLC grade acetonitrile analysis will be economical and also the injection of salt solution may not result in the phase separation of the organic phase in the mobile phase. The standard curve of DEC is linear in the range of 0.025–0.5 µg. Reproducible result was obtained up to a minimum concentration of 0.5 µg/ml. The precision of HPLC assay was tested at five different DEC levels over a range of 0.5–25 µg/ml. The recoveries of DEC added were more than 90% in all the cases and the coefficient of variation was below 10%.

In DEC-medicated salt programmes for the control of filariasis, DEC is mixed at 0.2% with the common salt. Comparing the quality of medicated salt obtained by the two methods of mixing, the data clearly show a higher and consistent level of DEC in samples prepared by spray drying method compared with the rotating drum method. Fan (1990) from Kinsmen Island reported that spray drying method gave a more uniform mixing. Reddy and Venkateswaralu (1996) reported that in a study conducted at

Table 2

Variation of DEC content in DEC-medicated salt samples prepared by two methods

Category	Level of DEC (%)	% of Samples	
		Spray drying method	Rotary drum method
Very low	<0.005	0	43.3
Low	0.005–0.15	0	35.8
Optimum	0.15–0.25	29	9
High	>0.25	71	12

Karaikal, South India where DEC mixing was done by rotating drum method gave a DEC content of 0.15–0.2% in 64.2% and < 0.15% in 25.6% of the medicated salt samples.

In the present study none of the samples from spray drying method was found to show either absence of DEC or the presence of DEC at very low level. Whereas majority of samples (43.3%) from rotating drum method showed either very low level or absence of DEC. This may be due to the improper mixing and less adhesion of DEC. In spray drying method, 29% of the samples were found to contain DEC at the level of 0.15–0.25% (optimum) and 71% of samples were found to contain DEC at > 0.25% level. Only 9 and 12% of samples from rotating drum method were found to contain DEC at the optimum level and slightly at a higher level, respectively.

The analytical method described here shows good reproducibility and permits easy analysis of a number of samples economically as the method does not require any sample extraction which is time consuming and cumbersome. Since the volume of the mobile phase is minimal, the cost of analysis is also minimal. The present method can detect a concentration of 0.5 µg/ml and, therefore, can be applied to situations even when the DEC content is 0.0005% as a result of mixing the salt with non-medicated salt by the vendor or the user. For the validation of the DEC medicated salt programmes as well as the quality checking of the DEC tablets, this method will be an additional support for the programme people. Thus, this quick and simple HPLC method for the estimation of DEC could play a vital role in checking the quality of the DEC medicated salt used for the control of filariasis.

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