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# COMMUNICATIONS

## The loss of paraben preservatives during freeze drying

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Recently the loss of two preservatives, methylparaben (MP) and propylparaben (PP) was encountered during freeze drying of an aqueous solution in our laboratory. Avis (1970) has noted that volatile antibacterial preservatives (benzyl alcohol, phenol and chlorobutanol) are not suitable for lyophilized products. However, we are unaware of any reports describing the loss of MP or PP during lyophilization. This report describes the factors influencing the loss of these antibacterial perservatives during freeze drying.

*Freeze-drying.* Solutions containing 0·8 mg ml$^{-1}$ MP (Amend Drug & Chem. Co., Inc., New York, NY), 0·2 mg ml$^{-1}$ PP (U.S.P.H.S. S.S.C., Perry Point, MD) and various amounts of mannitol (Aldrich Chem. Co., Milwaukee, WI) or sodium chloride in distilled water were prepared and dispensed (5·0 ml) into 10 ml flint vials. The vials were placed on the prechilled shelf (about − 40 °C) of pilot scale, 1 ft$^2$, freeze drying unit (Model 10–800, Virtus Co. Inc., Gardiner ,NY). After the contents of the vials were frozen, the product was processed through various drying cycles detailed in Table 1 and 2. Samples were removed and stored at − 15 °C until the completion of an experiment at which time all analyses were performed. Also, samples of one lot of calcium leucovorin (BV–79–205) an investigational product containing 4 mg MP and 1 mg PP per vial, were reconstituted with 5·0 ml of distilled water and freeze dried again in our pilot scale unit.

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*Analysis.* MP and PP were determined in the presence of mannitol or calcium leucovorin by reverse phase high pressure liquid chromatography (h.p.l.c.). MP, PP, internal standard (benzophenone) and calcium leucovorin were readily separated (retention volumes = 3·8 ml, 5·5 ml, 7·4 ml, respectively) on a C–18 (10 $\mu$m) column by elution with methanol–isopropanol–water (55 : 10 : 35, v/v) delivered at 1 ml min$^{-1}$. The eluted compounds were detected at 254 nm. Mannitol was not detected as it lacks u.v. absorbance. The freeze-dried samples were reconstituted for analysis with 5·0 ml of distilled water. A 125 $\mu$l aliquot was diluted to 10 ml with mobile phase containing internal standard, and a 10$\mu$ l aliquot was injected for h.p.l.c. analysis. Samples of calcium leucovorin (Ben Venue Labs., Bedford, Ohio, Lots BV–77–225, BV–78–255 and BV–79–205) were similarly diluted and analysed. These also contained 4 mg of MP and 1 mg of PP per vial.

The results in Table 1 indicate a substantial loss of both MP and PP during freeze drying in the pilot unit at 10–20 $\mu$m Hg. The losses became progressively greater as the drying time and product temperature increased. The paraben-containing calcium leucovorin product that was processed in a production freeze dryer was analysed and there was little or no apparent loss of MP or PP in the three lots. The content of MP and PP ranged between 97·2–99·7 and 98·2–98·3% of label, respectively. Freeze drying logs for typical batches indicate that the chamber pressure during the 62 h drying cycle varied from 90 to 200 $\mu$m Hg. An attempt

Table 1. Paraben content of products freeze-dried with vacuum of ~20 $\mu$m or 175 $\mu$m Hg

| Drying cycle (h) | Temp. Range C° * | % remaining (s.d.) ~20 $\mu$m MP | | n | PP | | ~175 $\mu$m MP | | n | PP | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 | − 30 to 0 | 95·7 | (3·7) | 12 | 95·9 | (2·8) | 98·4 | (0·6) | 6 | 99·7 | (1·8) |
| 24 | − 30 to 0 | | | | | | | | | | |
| 24 | 0 to 5 | 94·0 | (1·9) | 11 | 94·9 | (1·5) | 97·8 | (0·8) | 7 | 98·7 | (1·3) |
| 24 | − 30 to 0 | | | | | | | | | | |
| 24 | 0 to 5 | 58·1 | (3·2) | 10 | 66·5 | (2·5) | 90·0 | (1·4) | 7 | 93·7 | (1·2) |
| 24 | 5 to 25 | | | | | | | | | | |

* Temperatures were recorded at the beginning and at the end of each 24 h.

Table 2. Effect of weight of excipient on the loss of parabens

| Freeze drying Cycle | | Excipients* (mg of each component per vial) 90 mg Mannitol | | 45 mg Mannitol 45 mg NaCl | | 45 mg Mannitol | | 22·5 mg Mannitol | | No Excipients | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % remaining (s.d.) (n = 4) | | | | | | | | | |
| Temperature* | | | | | | | | | | | |
| h | Range (C°) | MP | PP | MP | PP | MP | PP | MP | PP | MP | PP |
| 24 | − 30 to 0° | 95·5 | 96·8 | 99·9 | 98·4 | 97·4 | 95·0 | 99·5 | 99·0 | 93·0 | 100·3 |
| 24 | − 30 to 0° | (2·4) | (2·6) | (0·5) | (1·5) | (0·4) | (2·6) | (0·2) | (2·2) | (2·3) | (2·4) |
| 24 | 0 to 25° | 78·6 | 81·1 | 77·1 | 78·5 | 70·7 | 71·7 | 75·7 | 79·5 | 67·0 | 73·7 |
| 24 | − 30 to 0° | (3·6) | (5·6) | (2·4) | (2·1) | (2·6) | (4·6) | (3·7) | (3·0) | (2·2) | (2·8) |
| 24 | 0 to 25° | 66·6 | 71·2 | 56·1 | 64·8 | 53·6 | 58·3 | 46·3 | 54·7 | 34·3 | 40·0 |
| 24 | 25° | (2·8) | (1·6) | (5·6) | (3·9) | (2·3) | (0·6) | (9·9) | (10·0) | (5·7) | (3·6) |

* All formulations initially contained 4 mg/vial MP and 1 mg/vial PP in addition to the excipients listed.
* Temperatures recorded at the beginning and at the end of each 24 h .

to reproduce a vacuum in this range in our freeze drying units using the mannitol-paraben product gave the other results seen in Table 1. Although the loss of MP and PP under these conditions was substantially less than observed at 10–20 $\mu$m Hg, some loss was evident. The reconstitution and subsequent repeat freeze drying at 10–20 $\mu$m Hg of the calcium leucovorin production lot (BV–79–205) resulted in the surprising retention of both MP (93·7%) and PP (95·9%). The drying cycle was identical to the longest cycle detailed at the foot of Table 2. Since the calcium leucovorin product contained 45 mg of sodium chloride in addition to the parabens and 50 mg of drug, the possibility that the increased bulk might account for the retention of the parabens was investigated. The data in Table 2 indicate that the total amount of other material present does reduce the loss of MP and PP. However, the effect is not enough to explain the retention of parabens in the calcium leucovorin product. A possible explanation can be seen in the u.v. spectra of a solution containing 10·9 $\mu$g ml$^{-1}$ calcium leucovorin, 2·2 $\mu$g ml$^{-1}$ MP and 0·55 $\mu$g ml$^{-1}$ PP. The $\lambda_{max}$ of calcium leucovorin is shifted 5 nm (from 283 to 278 nm) in the presence of parabens indicating the possible formation of a complex containing drug and parabens. Complexation between other compounds and hydroxybenzoates has been described previously (Poochikian & Cradock 1979; Higuchi & Kristiansen 1970; Lachman 1968). The reduced volatility of such a complex could explain the retention of the parabens in this product. Further support for this observation was sought with adriamycin hydrochloride, an antitumour agent supplied commercially as a freeze-dried product. This drug was chosen as a likely candidate based on physical and structural considerations (lipophilicity, planarity and aromaticity) to retain parabens by a similar mechanism. A formulation containing 10 mg adriamycin hydrochloride, 4 mg MP and 1 mg PP was freeze-dried in a pilot unit under the same conditions as described for the reconstituted calcium leucovorin product. Analysis indicated an average retention of 97·4% for MP and 95·4% for PP in the adriamycin product.

Although the loss of parabens was not evident in the calcium leucovorin or adriamycin product, the possibility that such a loss may occur with other drugs cannot be ignored. The results of this study show that the loss of MP and PP during freeze drying is dependent on the vacuum of the system, the length of drying cycle, the temperature of the product, and the amount and chemical entity of materials present. Analysis of paraben-containing freeze-dried products for both active ingredient and antibacterial preservatives appears warranted.

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