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International Journal of Pharmaceutics 340 (2007) 223–229
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Note

New analytical techniques to facilitate preformulation screening in propellant systems

Erik Mogalian *, Philip J. Kuehl, Paul B. Myrdal

College of Pharmacy, University of Arizona, Tucson, AZ, United States

Received 27 January 2007; received in revised form 16 March 2007; accepted 22 March 2007
Available online 25 March 2007

Abstract

The objective of these studies was to investigate the applicability of an online direct inject HPLC method for the preformulation screening of pharmaceutical agents in pressurized metered dose inhalers (MDIs). The technique was initially utilized for the solubility determination of solid solutes. This study explores the extension of the online direct inject method for the evaluation of drug stability in propellant systems as well as for the analysis of MDI vials crimped with metered valves. Through-life content analysis confirmed that a single vial may be repeatedly sampled, thus facilitating the stability evaluation of a single unit over time. The method was successfully used for evaluating the stability of a model drug, as a function of several different formulation configurations, with minimal sample numbers. Additionally, studies determined that after modifications were made to the injection coupler, the technique was also feasible for use with 50 and 100 μL metered valves, however further modifications are necessary for 25 μL valves.

Keywords: MDI; Preformulation; MDI stability; Metered valves; Direct inject; HFA

1. Introduction

Pressurized metered dose inhalers (MDIs) are currently the most common device utilized to deliver therapeutic agents to the lungs (Smyth, 2003). They have become a mainstay in the treatment of pulmonary diseases as they are inexpensive, reliable, and have the trust of both patients and physicians. Pressurized MDIs can either be formulated as suspension formulations or as solution formulations. In a suspension formulation, the drug particles are dispersed in the propellant system, while in a solution formulation the drug is dissolved in the propellant system with or without the need for the addition of different cosolvents.

In both types of formulation, the solubility of the therapeutic agents in the propellant system is vital. Specifically, in a suspension formulation, dissolved drug is susceptible to chemical degradation and may promote Ostwald ripening. In a solution formulation, drug solubility is important as it limits drug concentration in the formulation.

Previous studies have detailed methods to measure the solubility in a MDI (Dalby et al., 1991; Williams et al., 1999). The traditional method utilized to evaluate the solubility of an API in a pressurized metered dose inhaler is labor intensive, requires a large number of MDI vials and a great deal of time. This process involves one MDI vial per sample point, which must be compromised as the contents are analytically transferred to a volumetric flask, where the propellant is evaporated off. At this time, the API is reconstituted with an appropriate diluent and then assayed for content. Traini et al. (2006) recently described another method that does not require decrimping of the MDI vial. These methods, however, do not allow for direct injection of the MDI into the HPLC.

Gupta and Myrdal (2004a,b, 2005) have developed and tested a novel method for direct injection of a MDI into an HPLC. The new online direct inject method, schematic representation shown in Fig. 1, allows for the direct injection of an MDI into the manual inject port of the HPLC. This method potentially provides numerous advantages over the traditional method in analysis of MDIs. These advantages include decreased number of sample vials, decreased sample preparation time and the ability to acquire multiple sample points from one MDI vial (Gupta and Myrdal, 2005).

* Corresponding author at: Department of Pharmacy Practice and Science, College of Pharmacy, 1703 E. Mabel St., Tucson, AZ 85721-0207, United States. Tel.: +1 520 626 3847; fax: +1 520 626 4063.
E-mail address: mogalian@pharmacy.arizona.edu (E. Mogalian).

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doi:10.1016/j.ijpharm.2007.03.026

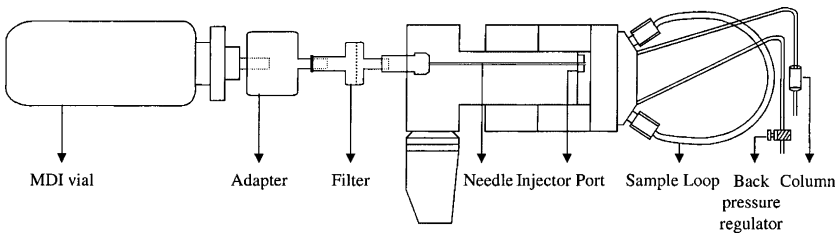

Fig. 1. Schematic representation of the online direct inject method.

Characterizing the degradation of an API in different formulations is obligatory in early formulation screening. In order to determine the stability of an API in an MDI, the methods of Dalby et al. (1991) and Williams et al. (1999) are utilized to quantify the amount of API at a given time. These methods are repeated on separate MDI vials over time to characterize the degradation of the API. Conducting stability studies in this manner requires that the laborious process be repeated numerous times on a large number of MDI vials. Logically, the online direct inject method would provide several advantages when conducting stability studies in an MDI; specifically, decreased number of vials, decreased amount of API and other formulation components required, and a significant decrease in the amount of time required for analysis.

As such, one of the focal points of this work was to evaluate the utility of the online direct inject method for characterizing the stability of an API in an MDI. For this evaluation, Imexon was chosen as the model drug. Imexon, Fig. 2, was selected as its stability has been well characterized in aqueous environments and analytical methodologies have been established (Kuehl et al., 2006).

Traditional methods detailed above allow for quantitative analysis of MDIs crimped with either metered or continuous valves, as the MDIs are ultimately decrimped prior to analysis. Currently, the online direct inject method has only been evaluated for use with MDIs crimped with continuous valves. The second focus of this study was to extend the applicability of this online direct inject HPLC method to include analyzing drug content of inhalers crimped with metered valves. As the original validation of the online direct inject method was conducted with beclomethasone diproprionate (BDP) these studies will similarly utilize BDP (Fig. 3).

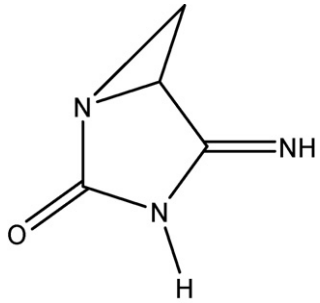

Fig. 2. Chemical structure of Imexon.

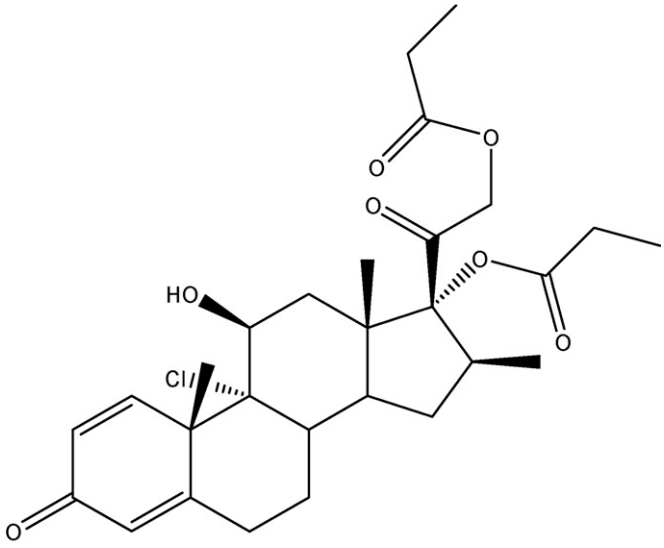

Fig. 3. Chemical structure of Beclomethasone dipropionate (BDP).

2. Materials and methods

2.1. Through-life analysis

2.1.1. Materials

Beclomethasone dipropionate and continuous valves were provided by 3M Drug Delivery Systems (St. Paul, MN, USA). Pressure resistant glass aerosol vials were purchased from Research Products International Corporation (Mt. Prospect, IL, USA). 1,1,1,2-Tetrafluoroethane (HFA-134a) and ethanol (200 proof) were obtained from DuPont Chemicals (Wilmington, DE, USA) and Aaper Alcohol and Chemical Company (Shelbyville, KY, USA), respectively. HPLC grade acetonitrile (ACN) was obtained from EMD (Gibbstown, NJ, USA). A Millipore (Billerica, MA, USA) Milli-Q Ultrapure Water purification system with a 0.22 μm filter was utilized for water.

2.1.2. Methods

Through-life analysis was conducted using a solution MDI containing 0.1% (w/w) BDP, 9% (w/w) ethanol, and HFA-134a, crimped with a continuous valve. Canister weight was measured before and after each injection, and HPLC measurements were taken throughout the life of the canister (21.2 g formulation). The online direct inject method was employed to allow for direct injection of the MDIs into a Waters 600E multisolvent delivery module (Waters, Milford, MA, USA) coupled

with a Waters 2487 diode array (Dual) detector. Analysis was performed by reverse phase HPLC, using a 150 mm × 4.6 mm Apollo C_{18} 5 μ column (Alltech Associates, Deerfield, IL). Ultraviolet detection was set at 240 nm. Mobile phase consisted of 90:10 (v/v) ACN:H_2O at a flow rate of 0.6 mL/min. Injection volume was 5 μL. BDP exhibited a retention time of 5.2 min.

2.2. Stability study

2.2.1. Materials

Imexon was provided by AmpliMed Corp., Tucson, AZ, USA. Valves were provided by 3M Drug Delivery Systems (St. Paul, MN, USA) and pressure resistant glass aerosol vials were purchased from Research Products International Corporation (Mt. Prospect, IL, USA). Ammonium acetate was obtained from Sigma–Aldrich (St. Louis, MO, USA). All other materials were as described in the Through-Life Analysis materials section.

2.2.2. HPLC method

The online direct inject method was employed to allow for direct injection of the MDI into a Waters 600E multisolvent delivery module (Waters, Milford, MA, USA) coupled with a Waters 2487 diode array (Dual) detector. Analysis was performed by a normal phase HPLC assay, using a 150 mm × 4.6 mm Apollo Silica 5 μ column (Alltech Associates, Deerfield, IL). Ultraviolet detection was set at 234 nm. Mobile phase consisted of 90:10 (v/v) ACN:H_2O at a flow rate of 0.6 mL/min. Water was buffered with ammonium acetate at 0.1 M, with a pH of approximately 6.5. Injection volume was 5 μL. The parent compound had a retention time of 7.8 min.

2.2.3. Solubility of model drug, Imexon

The solubility of Imexon was determined in pure HFA-134a propellant at 23 °C. The solubility of Imexon was also determined as a function of the cosolvent ethanol at 5, 10, 15, 20 and 25% (w/w). Preformulation work has also shown that Imexon has favorable water solubility (∼25 mg/mL, at 23 °C), thus the solubility of Imexon was also explored as a function of water. Using ethanol as a cosolvent for water, solubility was determined for formulations containing 0.5 and 1% H_2O (w/w) with 10% EtOH, and 0.5, 1, 1.5, 2, and 3% H_2O (w/w) with 20% ethanol (w/w).

2.2.4. Stability of model drug, Imexon

The stability of Imexon in an MDI environment was measured as a function of temperature at 11, 23 and 37 °C, with a MDI containing 80:20 HFA-134a:EtOH and 80 μg/g of Imexon. The effect of EtOH was evaluated at 80:20 and 75:25 HFA-134a:EtOH with a drug concentration of 80 μg/g. The stability as a function of H_2O was determined with compositions of 80:20:0, 79:20:1 and 78:20:2 (HFA-134a:EtOH:H_2O, % w/w/w) with 80 μg/g. Because initial drug concentration had an effect on stability in aqueous studies, the effect of initial drug concentration on stability was determined at concentrations of 80, 150 and 250 μg/g with an inhaler composition of 78:20:2 (HFA-134a:EtOH:H_2O, % w/w/w). Four injections were used for each sample condition.

2.3. Metered valve study

2.3.1. Materials

All materials were obtained as described in Section 2.1.1.

2.3.2. Experimental method

A stock solution formulation (0.078% w/w BDP, 9% w/w ethanol, HFA-134a) was prepared, chilled, and then transferred into 8 glass vials via cold transfer. These eight inhalers were crimped (two each) with continuous-flow valves or with 25, 50, or 100 μL metered valves.

Each inhaler was assayed using the online direct inject HPLC setup as mentioned in Section 2.1.2 above, however, alterations were made to the stem-needle juncture. The adapter and filter were eliminated to decrease losses upon actuation as seen in Fig. 4. A pre-column filter was used to capture any particulate matter from the inhalers in place of the removed filter. In order to create a seal between the valve-stem and injection needle, rubber o-rings were used. These changes were done to decrease the internal volume of the system to allow for analysis small amounts of (metered) formulation. Four injections were measured for each inhaler.

Fig. 4. MDI with the traditional injection apparatus (left), and modified injection coupler (right).

3. Results and discussion

3.1. Through-life analysis

Single HPLC measurements were taken for the life of an inhaler and are shown in Fig. 5. On average, each injection used 580 mg of formulation, which correlates to 30+ possible injections for every 20 g of formulation. This was calculated using the average weight emitted over the series of consecutive injections (from ~12–20 g formulation used). Because no significant deviations were expected early in the life of the inhaler, multiple actuations were sampled to waste, accounting for the gaps noted in Fig. 5. Percent of theoretical concentration did not appear to change as a function of formulation used and is thus of limited concern when repeatedly sampling an MDI vial. Of note is that this analysis was conducted in a single day, subsequently propellant leak over time may be a factor to consider, as in the case of stability studies. Additionally, care should be taken to avoid assaying canister contents when minimal formulation remains, as end-of-life issues are documented with MDIs (Olley, 2007; Cripps et al., 2000).

3.2. Solubility of the model compound, Imexon

Based on aqueous preformulation studies of Imexon, several factors contributed to the stability of the compound, including temperature, water, ethanol, and initial drug concentration (Kuehl et al., 2006). As such, those factors were examined over time in the MDI environment using the online direct inject HPLC method for analysis. Prior to evaluating the stability of the model drug in a MDI, the solubility of Imexon was determined as a function of several different variables.

The solubility of Imexon in pure HFA-134a was found to be 0.00022% (w/w). The solubility of Imexon as a function of the cosolvent EtOH is presented in Fig. 6. As the figure indicates, the solubility of Imexon increases linearly as a function of EtOH.

Given that Imexon is relatively polar, the use of water to increase the solubility of Imexon was also evaluated. However, in view of the fact that water is relatively immiscible with HFA-134a alone, the presence of either 10 or 20% (w/w) ethanol was incorporated to solubilize the water. Fig. 7 displays the solubility increase for Imexon with 10% ethanol and water concentrations of 0, 0.5, and 1% (w/w) as well as for 20% ethanol with 0, 0.5, 1, 1.5, 2, and 3% (w/w) water concentrations.

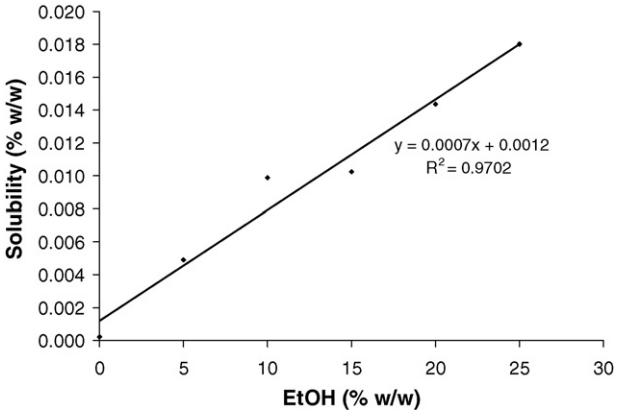

Fig. 6. Solubility of Imexon in a MDI as a function of EtOH in HFA-134a.

The solubility of Imexon increases linearly as a function of H_2O concentration for both ethanol concentrations.

These solubility data, along with preformulation data collected under aqueous conditions, were utilized to determine the formulation combinations for evaluating the utility of the direct inject method for chemical stability. Namely, these were the effect of temperature, H_2O, EtOH and initial drug concentration on the degradation of Imexon in a pMDI.

3.3. Stability of the model compound, Imexon

Prior to formulation of stability vials, the effectiveness of the direct inject method to function as a stability indicating method was established. The HPLC conditions (column, mobile phase, flow rate, etc.) previously used to characterize the stability of Imexon under aqueous conditions were combined with the direct injection method (Kuehl et al., 2006). Importantly, the mobile phase contains a high percentage of organic (90:10, ACN:H_2O) which facilitates the direct injection of the non aqueous formulation (Gupta and Myrdal, 2004b). For an initial screen, a formulation was prepared (80:20 HFA-134a:EtOH, 80 μg/g)

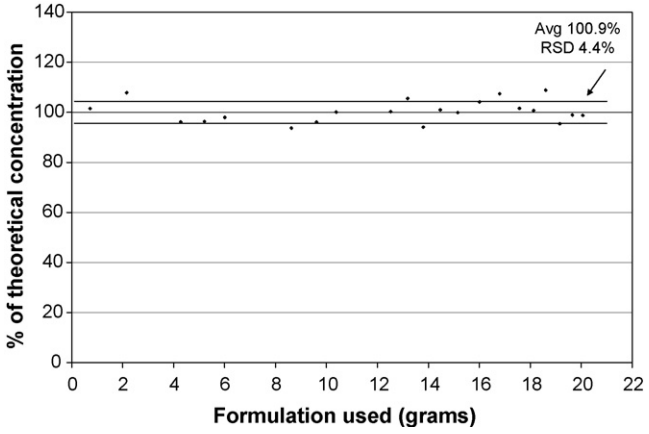

Fig. 5. Through-life concentration analysis of an inhaler, 21.2 g total formulation.

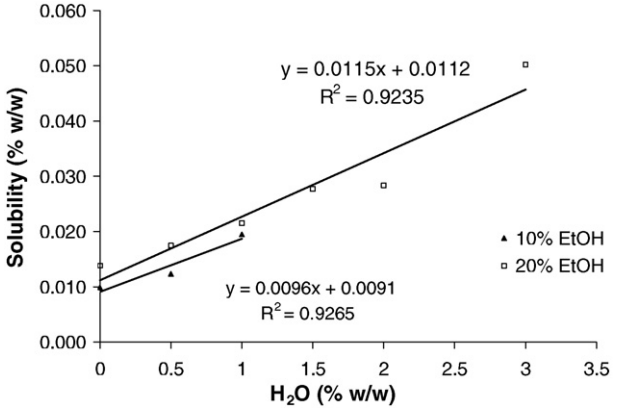

Fig. 7. Solubility of Imexon as a function of water with 10 and 20% EtOH in HFA-134a.

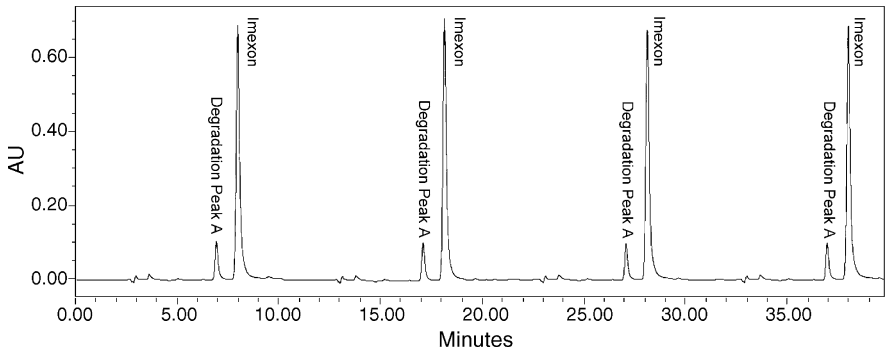

Fig. 8. Representative chromatograms of an Imexon stability sample showing the separation of a degradation product ($n = 4$ injections).

and crimped with a continuous valve. The vial was stored at 37 °C to facilitate the degradation of the Imexon parent drug. The chromatography of the Imexon and degradant from the non aqueous system was similar to that observed from analysis of Imexon under aqueous conditions. As can be seen from the four sequential injections represented in Fig. 8, the Imexon peak is well resolved from Degradation Peak A. The degradation product is proposed to be the same degradation product observed in aqueous media (Kuehl et al., 2006; Den Brok et al., 2005a,b).

The overall method capabilities were in alignment with aqueous analysis, having a linear range from 5 to 500 μg/mL. Collectively, repeated analyses from individual vials were found to afford a R.S.D. of 4% (under all conditions tested). From these data it was concluded that the direct inject method is feasible for use in preformulation stability studies. In order to accurately determine the concentration at each time point, each vial was injected four times. The reported concentrations are averages of these four injections.

3.3.1. Effect of temperature

The effect of temperature on the degradation of Imexon was assessed with formulations conditions of 80:20 (HFA-134a:EtOH), 80 μg/g initial Imexon concentration stored at three different temperatures (11, 23 and 37 °C). To maintain consistency, the inhalers were brought to room temperature for analysis and then immediately returned to the appropriate storage condition. Analysis of Log percent drug remaining as a function of time (Fig. 9) indicates that Imexon undergoes apparent 1st order degradation in an MDI environment, which was ultimately observed to be the same for all MDI conditions evaluated. These studies were conducted over a period of ∼3 months. During that time, the most degradation Imexon went through was ∼2 half-lives; however, due to the log-linear nature of the degradation, they were identified as apparent 1st order processes.

Analysis of the effect of temperature on the degradation of Imexon indicates that the degradation rate of Imexon increases as the temperature increases. An Arrhenius plot for the three temperatures evaluated is shown in Fig. 10, which results in a calculated activation energy for Imexon of 110.04 kJ/mol in the propellant system. Additional relevant degradation parameters for all conditions evaluated in a MDI are shown in Table 1 (A–C).

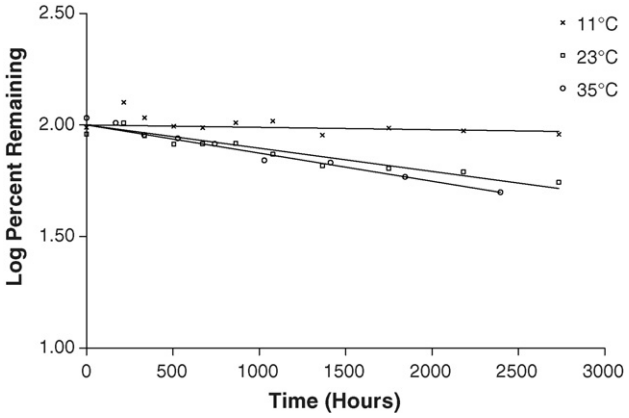

Fig. 9. Log percent remaining for Imexon in a propellant system, 80:20 (HFA-134a:EtOH) with 80 μg/g Imexon, stored at either 11, 23 or 35 °C, indicating 1st order degradation.

3.3.2. Effect of EtOH

The effect of EtOH concentration on the degradation of Imexon in an MDI environment was evaluated at EtOH concentrations of 20 and 25% (w/w). Referring to Table 1 (B, H), it can be seen that an increase in EtOH concentration decreases the degradation rate of Imexon.

3.3.3. Effect of H_2O

In order to elucidate the effect water concentration has on the degradation of Imexon in a MDI, formulations were prepared

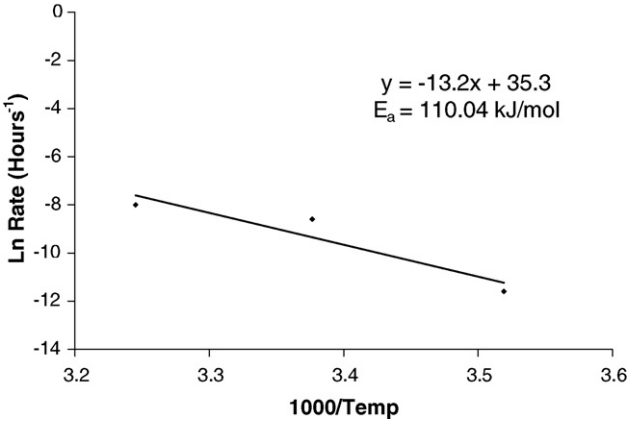

Fig. 10. Arrhenius plot for Imexon in an MDI environment.

Table 1
Calculated degradation parameters for Imexon in an MDI environment

Row #	HFA-134a (%)	EtOH (%)	H_2O (%)	Imexon (μg/g)	Temperature (°C)	k (h^{-1})	T_{50} (h)	T_{95} (h)
A	80	20	0	80	11	0.000009	75228	5591
B	80	20	0	80	23	0.000184	3761	279.5
C	80	20	0	80	37	0.000336	2061	153.2
D	79	20	1	80	23	0.000576	1204	89.4
E	78	20	2	80	23	0.000649	1067	79.3
F	78	20	2	150	23	0.000415	1672	124.2
G	78	20	2	250	23	0.000394	1760	130.8
H	75	25	0	80	23	0.000157	4425	328.9

with water concentrations of 0, 1 and 2% (w/w). As would be expected based on aqueous preformulation stability, an increase in the concentration of water resulted in an increase in the degradation rate of Imexon as can be seen in Table 1 (B, D, E).

3.3.4. Effect of initial drug concentration

Under aqueous conditions the initial concentration of Imexon was determined to have a direct correlation to the degradation rate (Kuehl et al., 2006). In order to determine if this was true in an MDI, formulations were prepared at 80, 150 and 250 μg/g. Analysis of these formulations indicates that when the concentration is increased the degradation rate is decreased as can be seen in Table 1 (E–G).

Overall, these data not only describe the stability of the model drug, as a function of several different formulation variables, they more importantly establish the ability of direct inject method to determine the stability in a non aqueous propellant system. Specifically, the data collected for the model drug indicated that Imexon displayed first-order degradation for all variables tested, and the degradation product was chromatographically resolved from the parent compound just as it was in aqueous studies.

It is important to note that these data were conducted with 8 MDI vials, assayed four times at each time point. As each vial was sampled at approximately eight different time points, these 8 MDI vials were utilized to generate over 250 data points. Collecting similar data with the traditional method (Dalby et al., 1991) would have required one MDI vial for each data point, or in excess of 250 MDI vials. This reduction in the number of vials required affords a considerable decrease in material and drug supplies, as well as a significant reduction in analyst time.

3.3.5. Metered valve study

The previously established direct inject method allows for the analysis of MDI content when crimped with a continuous valve (Gupta and Myrdal, 2004a,b, 2005). This portion of the study is intended to evaluate the utility of the method to include analysis of inhalers crimped with different sizes of metered valves.

The traditional method utilizes an adapter and filter between the stem and injection needle, as can be seen in Fig. 1. However, upon analyzing the canister contents using the traditional setup of the direct inject method, the MDIs crimped with a 25 μL valve did not achieve the desired content, resulting in 12.5% of the theoretical concentration. The vials crimped with 50 and 100 μL valves were comparatively improved but still not adequate, resulting at 55.8% and 82.5% of theoretical concentration, respectively. As such, it was clear that the losses from the traditional setup were too great and thus the external filter was eliminated. With the filter removed, there was no longer a need for an adapter as the injection needle fits the stem of the MDI with the help of rubber o-rings to seal the juncture. Fig. 4 shows the traditional setup (including filter and adapter) and the modified setup.

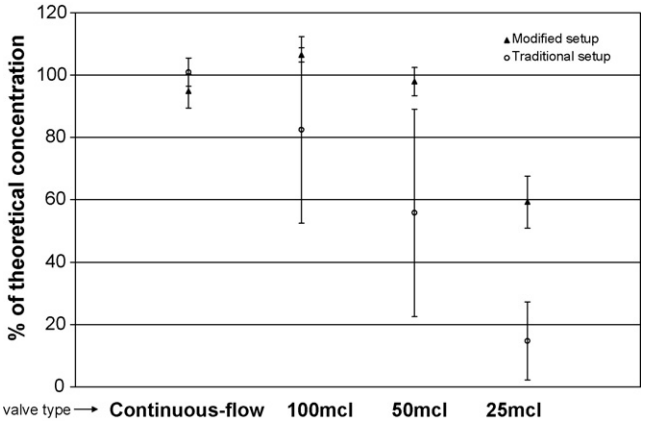

Fig. 11. Comparison of the traditional and modified setup for different valve types.

Once the injection coupler was modified, measurements from MDIs with 50 and 100 μL metered valves compared favorably to the vial with a continuous-flow valve in both concentration and variability. Fig. 11 shows the results of using the traditional setup compared to the modified setup. The MDI with a 25 μL was relatively improved with the modified setup, however, was still only at 60% of the theoretical concentration, indicating that even with the reduced adapter volume, there was not ample formulation for loop filling.

Though the changes made to the system did allow for the analysis of contents from inhalers with larger metered volumes (50 and 100 μL), engineering an injector needle specifically for this purpose would likely improve the variability and possibly improve the efficiency such that analysis of inhalers crimped with 25 μL metered valves would be possible.

4. Conclusions

Through-life analysis indicates that an API, in a single vial with a continuous valve, can be repeatedly analyzed via the

direct inject method (30+ samples per 20 g of formulation). Solubility analysis, as previously described using this method, was performed on the agent Imexon. Stability studies conducted on Imexon indicate that the online direct inject method is a viable and resource conserving analytical method (using 8 versus 250+ MDI vials) for determining chemical stability as a function of several different formulation factors. Specifically, they were used to determine that Imexon undergoes apparent 1st order degradation under all propellant conditions evaluated. The degradation rate of Imexon in a MDI is increased by increasing temperature and concentration of H_2O, while increasing EtOH or initial drug concentration appears to decrease degradation rate.

Alterations to the injection coupler allowed for accurate content analysis of MDIs crimped with metered valves of larger volumes (50 and 100 μL), however it was not efficient enough to accurately analyze inhalers crimped with 25 μL valves. With improvements to the engineering of the injection coupler, it should be possible to accurately analyze the range of commercially available metered valves. In summary, it has been found that the direct inject method can be a useful technique for preformulation screening in propellant based systems.

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