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**Article**

# Powerful and Fast Structural Identification of Pharmaceutical Impurities using Direct Injection Mass Spectrometry and Differential Scanning Calorimetry

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In this work, direct injection mass spectrometry with electrospray positive ion mode has been tested to confirm the possibility of contamination initially detected in loratadine raw material through visual evaluation and differential scanning calorimetry (DSC). MS and MS/MS experiments were performed using a Waters TQD Acquity instrument with an electrospray ionization source with automatic injector. DSC analysis allowed us to confirm the difference in the thermal profile of loratadine raw material and the material of pink appearance, then to corroborate the visual inspection of the raw materials indicating contamination in the loratadine raw material. Pink raw material was submitted to extraction diluted to 10 μg $mL^{-1}$ in acetonitrile/water (50:50) with 0.05% formic acid and directly injected to the mass spectrometer (DI-MS). In the single mass spectrum of the pink compound it was possible to verify the presence of the signal at *m/z* 383 of protonated loratadine $[M+H]^+$ and the impurity signal at *m/z* 865 which was studied by tandem mass spectrometry to access its structural features. Collision-induced dissociation of the ion of *m/z* 865 resulted in product ions allowing the proposition of two similar structures: the formation of charge transfer complex between loratadine (*m/z* 383) and a known impurity of loratadine (*m/z* 482/483) or an ion/molecule reaction producing a covalently bonded compound.

**Keywords:** Loratadine, direct injection analysis mass spectrometry, differential scanning calorimetry, DSC, active pharmaceutical ingredients, API, impurity profile.

## INTRODUCTION

Classification and identification of impurities in raw material is essential to the quality control of chemicals in the pharmaceutical industry. Raw materials of drugs are the most important class of chemicals to be evaluated. On their purity depends the pharmacological effect inherent in pharmaceutical formulations. Qualification of those impurities as present or not present in a raw material demands examination of chemical aspects that involve instrumental analysis. Impurities can usually be classified as organic, inorganic, or residual solvents [1-2].

Organic impurities (OI) are unwanted substances that are sometimes formed during the manufacturing process and storage. OI identification should be carried out to avoid collateral effects that can put in danger people taking the drugs. OI molecules identified can arise from starting materials, by-products, intermediates, degradation products, reagents, ligands and catalysts [1-2].

Concerns over safety of pharmaceutical products and impurity profiles are of increasing importance in drug development and regulatory assessment. Usually most active pharmaceutical ingredients (API) are manufactured by organic chemical syntheses and the major step where the impurity may be produced or observed is in the organic synthesis of the target molecule. Impurities may arise from the starting reagents or from byproducts or degradation during chemical transformations [3-4].

Several components can be formed during such a process. Those components remaining in the final API raw material are considered as impurities. The sources of and routes to the formation of impurities

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involve several aspects that require specific assessments for each drug and finished product, such as heat, light, oxidants, changes in the pH of the formulation, trace metal impurities and interactions with packaging components, excipients and other active pharmaceutical ingredients. The degradation reactions that may occur are hydrolysis, oxidation, and photolytic cleavage [5].

Physical properties have a strong impact on a material's bulk properties, product performance, stability and product appearance. Characteristics of a substance that can be realized with the senses are called organoleptic properties, such as physical state (color, odor, transparency and brightness). Melting point, chemical reactivity, apparent solubility, dissolution rate, optical and mechanical properties, vapor pressure and density can all be affected. Understanding the physical and chemical properties of the solid pharmaceutical materials might increase the chances of successful drug product and process development [6-9].

There are several situations in pharmaceutical chemistry investigations that can be solved by understanding physical properties measured in macroscopic ways; however, for further development to obtain molecular information and to solve complex problems, the mass spectrometry technique appears to be a strong tool and performs best when linked to MS and MS/MS equipment. Batch analysis of the pharmaceutical product or raw API for product release may require long analysis times. However, electrospray ionization mass spectrometry (ESI-MS) molecular fingerprinting analysis has been demonstrated to be very effective in the rapid structural elucidation of pharmaceutical impurities [10-16].

In this work, mass spectrometry with electrospray positive ion (ESI+) mode has been tested to confirm the possibility of contamination initially detected in loratadine raw materials through visual evaluation of their appearance and Differential Scanning Calorimetry (DSC). The positive mode was used because loratadine is a drug with basic characteristics due to the presence of the nitrogen atom. Consequently, the best analytical condition for this compound is after protonation with formic acid. This result in a more efficient ionization, with better sensitivity to analysis in the ESI+ mode, allowing a greater intensity to evaluate the structure of the formed impurity that possibly also presents basic characteristics. Since the impurity was successfully characterized in positive ion mode the use of negative ion mode was not chosen.

## MATERIALS AND METHODS

### *Reagents*

Acetonitrile, formic acid and methylene chloride (analytical grade) were obtained from Merck (Darmstadt, Germany) and 2-(2-ethoxyethoxy)ethanol (Transcutol®) was purchased from Gattefosse SA. (Saint Priest, France). Water was purified using Milli-Q Advantage A10 System.

### *Instrumentation*

Differential screening calorimetry (DSC) measurements were performed using a Mettler Toledo instrument (Switzerland). The samples were weighed directly in pierced aluminum pans (2.5 - 5 mg) and scanned between 25 ºC and 350 °C at a heating rate of 10 °C $min^{-1}$ under a nitrogen flow of 80 mL $min^{-1}$.

MS and MS/MS experiments were performed using a Waters TQD Acquity/Micromass UK Limited mass spectrometer with an electrospray source (Manchester, England).

LC System equipped with a binary pump was connected to an autosampler (Milford, USA).

### *Direct injection mass spectrometric condition*

The mobile phase consisted of acetonitrile/water (60:40) with 0.05% formic acid while the diluent was acetonitrile/water (50:50) with 0.05% formic acid. The flow rate was 0.1 mL $min^{-1}$ and the temperature of the autosampler was maintained at 15 °C. The DI-MS and DI-MS/MS parameters are presented in Table I.

**Table I.** DI-MS and DI-MS/MS parameters.

| Acquisition Mode | Full Scan | Daughter Scan |
|---|---|---|
| Polarity | ES+ | ES+ |
| Capillary (kV) | 3.00 | 3.00 |
| Cone (V) | 30.00 | 30.00 |
| Extractor (V) | 3.00 | 3.00 |
| RF (V) | 0.60 | 0.60 |
| Source Temperature (°C) | 120 | 120 |
| Desolvation Temperature (°C) | 500 | 500 |
| Cone Gas Flow (L $h^{-1}$) | 25 | 25 |
| Desolvation Gas Flow (L $h^{-1}$) | 500 | 500 |
| Collision Gas Flow (mL $min^{-1}$) | 0.10 | 0.10 |
| LM 1 Resolution | 10.00 | 15.00 |
| HM 1 Resolution | 10.00 | 15.00 |
| Ion Energy 1 | 0.50 | 0.50 |
| LM 2 Resolution | 10.00 | 15.00 |
| HM 2 Resolution | 10.00 | 15.00 |
| Ion Energy 2 | 0.50 | 0.50 |
| MS Mode Entrance | 50.00 | 1.00 |
| MS Mode Collision Energy | 3.00 | 10.00 |
| MS Mode Exit | 50.00 | 0.50 |

## RESULTS AND DISCUSSION

Loratadine {Ethyl-4-[8-chloro-5,6-dihydro-11H-benzo(5,6)cyclohepta(1,2-b)pyridine]-1-piperidine carboxylate} is a second-generation, non-sedating, long-acting antihistamine which is employed in the symptomatic relief of allergies such as hay fever, urticaria and seasonal allergic rhinitis and it elicits this effect by selective and peripheral antagonistic action on histamine-1 receptors [17-18].

The regular appearance of loratadine raw material is white, but, when visually analyzing a sample of loratadine raw material from a different provider, it was found to be a pink color (Figure 1) not in accordance with the expected appearance, leading to further investigation.

**Figure 1.** Photo of a loratadine sample showing pink color (left) and another sample of loratadine with white color (normal appearance) (right).

First investigation started with a solubility test in 2-(2-ethoxyethoxy)ethanol (Transcutol®) of a regular loratadine sample and the pink sample, since regular loratadine is not water-soluble. In solution the pink color was better observed while the regular loratadine with white appearance resulted in a clear transparent solution (Figure 2).

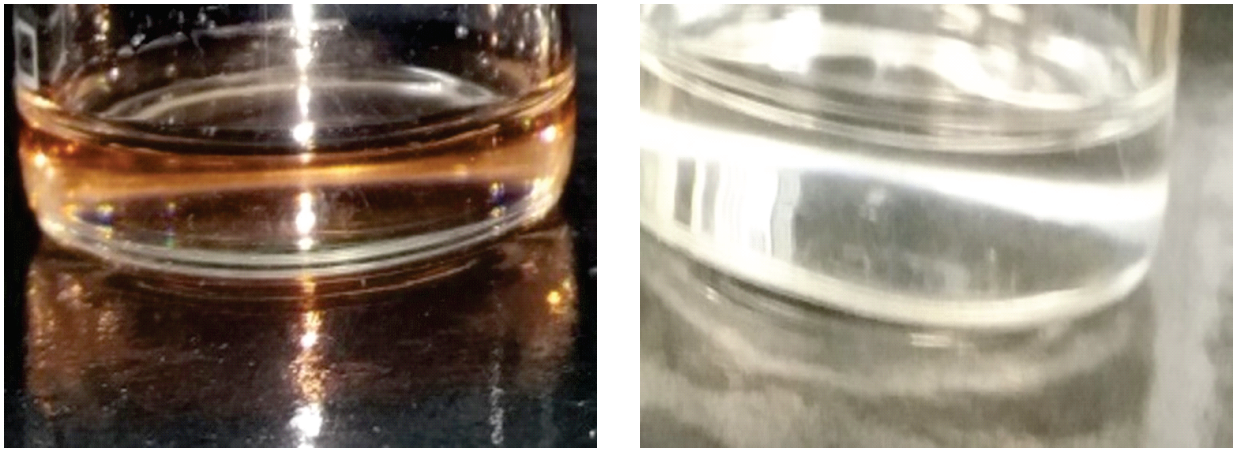


**Figure 2.** Photo of loratadine raw material presenting pink color (left) and white color solubilized in Transcutol® (2-(2-ethoxyethoxy)ethanol) (right).

Differential Scanning Calorimetry (DSC) was used to compare the difference in the thermal profile of the raw material of loratadine with normal appearance and the sample of loratadine with pink appearance. Figure 3 shows contamination in the investigated raw material of loratadine (pink).

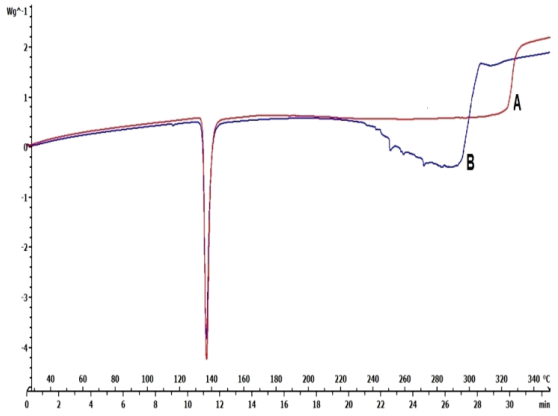



**Figure 3.** DSC of loratadine with (A) white appearance and (B) pink appearance.

DSC analysis gave results that confirmed the visual observation of an impurity in the pink material, but the identification was not achieved. To do that it was necessary to extract the pink impurity to perform better identification. First the acid-base properties of loratadine were studied, and using the Henderson-Hasselbach equation [Ionization (%) (α) = 100 − 100 / 1 + antilog (pKa – pH)], with pKa = 5.0 at pH 1.2, giving ionization of 99.9%, led to the conclusion that almost 100% is ionized at pH 1.2 in aqueous media [19].

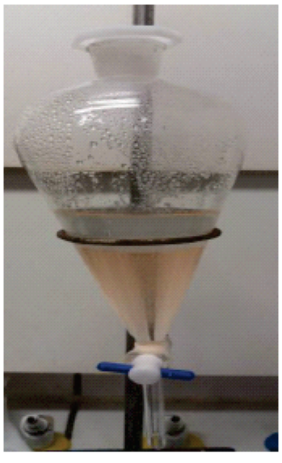


**Figure 4.** Extraction of loratadine sample with pink appearance.

After the extraction process the organic phase was evaporated and the DSC analysis of the resulting powder was performed. Overlaying DSC results of purified pink compound with DSC results of pink loratadine sample it was possible to confirm the characteristics of the contaminant in the raw material. The active pharmaceutical ingredient loratadine has a melting point of approximately 140 ºC whereas the pink compound had a melting point of approximately 125 ºC (Figure 5). A plausible explanation would be that although the impurity has a higher molecular mass, it would be less closely packed in its crystalline structure; that is, it has a lower intermolecular interaction and consequently a lower melting point than the loratadine molecule.

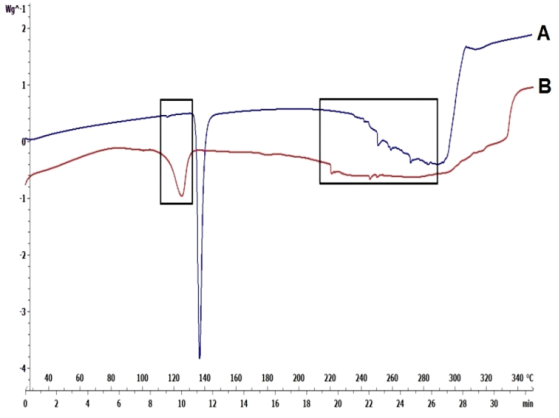



**Figure 5.** DSC of (A) loratadine sample with pink appearance and (B) its isolated impurity.

The final part of the impurity evaluation was the molecular analysis using direct injection mass spectrometry. Solutions of 10 μg $mL^{-1}$ in acetonitrile/water (50:50) with 0.05% formic acid of the pink isolated impurity and the regular white loratadine raw material were injected in positive ion electrospray ionization (ESI+) mode. Figures 6 and 7 show the results of the injections for the pink isolated impurity and the regular white loratadine raw material.

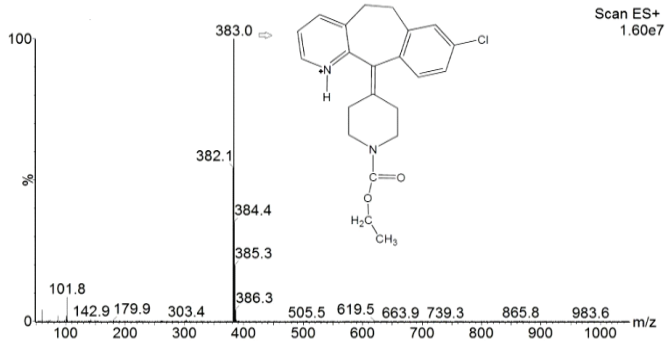



**Figure 6.** Mass Spectrum scanning of loratadine raw material with white appearance.

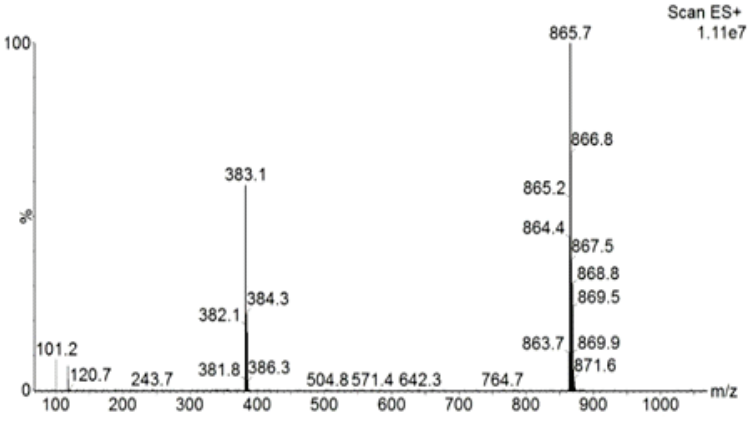



**Figure 7.** Mass Spectrum scanning of isolated pink compound.

We might notice in MS spectrum (Figure 6) the predominant signal of the ion of *m/z* 383 from protonated loratadine ($[M+H]^+$) and (Figure 7) signals at *m/z* 383 from protonated loratadine and of *m/z* 865 from the pink impurity which might to be the contaminant present in the pink loratadine raw material. The origin of the ion of *m/z* 383 in the isolated pink compound MS is from trace amounts of loratadine solution because the extraction process was not fully efficient.

A literature survey reveals that drug substances and drug products are routinely analyzed for impurities and related substances and for assay of active pharmaceutical ingredient (API) content to ensure efficacy and safety of the pharmaceutical product. One of the simplest methods of drug analysis is the formation of charge transfer complexes between the drug acting as electron donor and various electron-deficient reagents acting as electron acceptors. Molecular interactions between electron donors and acceptors are generally associated with the formation of intensely colored charge-transfer complexes [20-22].

In this study it was possible to identify that the color change of loratadine raw material is the formation of a charge-transfer complex. Tandem mass spectrometry experiments were carried out to access information about the structure of the impurity candidate of *m/z* 865.

Figure 8 shows results of a collision-induced dissociation experiment selecting in the first quadruple the ion of *m/z* 865 and colliding with argon at 15 eV in the collision cell, and scanning the last quadrupole mass analyzer from *m/z* 50 to *m/z* 900 mass range.

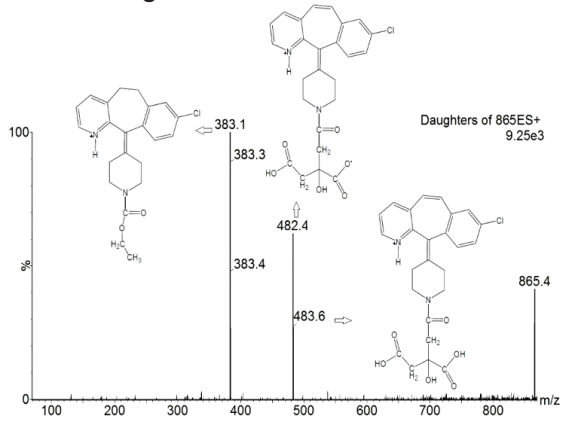
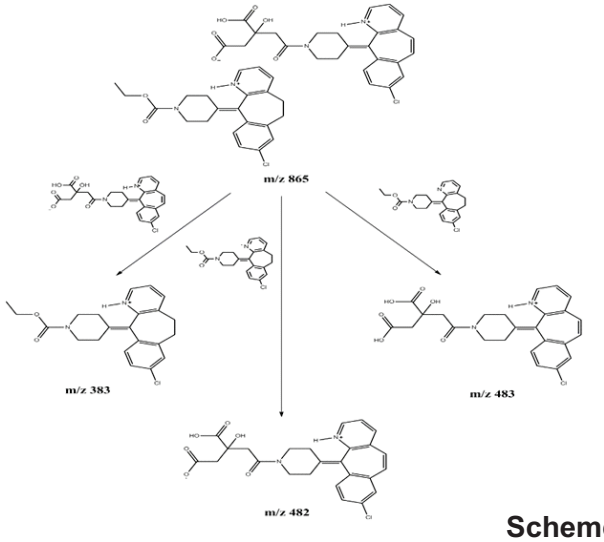



**Figure 8.** Mass Spectrum fragmentation of *m/z* 865 ion.

Fragments of *m/z* 483 and *m/z* 383 suggest a precursor ion formed by a proton dimer of loratadine and the impurity, and we suggest the ion of *m/z* 482 is the molecular ion of the impurity resulting from an electron transfer from the zwitterionic impurity part to the protonated loratadine, as shown in Scheme 1.




**Scheme 1**

The charge transfer complex (of *m/z* 865) dissociates to generate a distonic ion of *m/z* 482 (open shell species), which is a perfectly plausible phenomenon to occur in gas phase ionization [23]. Figure 9 shows the suggested structure for the impurity as a charge transfer complex that seems to be corroborated by the tandem mass experiment.

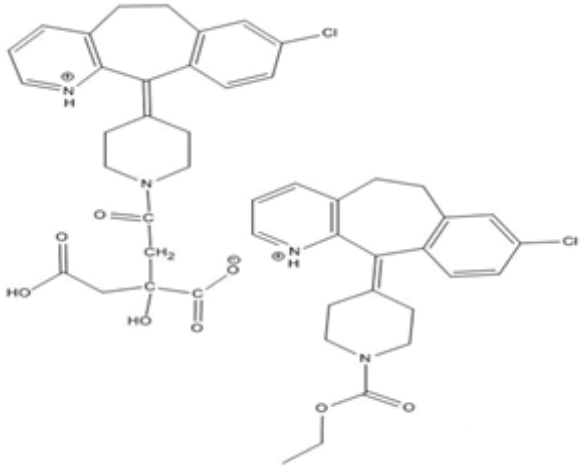


**Figure 9.** Suggested structure of charge-transfer complex.

Another possible structure for the formation of pink impurity with *m/z* 865 in the sample of loratadine raw material would be a covalent bond between the negative oxygen of the known impurity and the electrophilic carbon connected to the nitrogen of the loratadine molecule, formed by an ion/molecule reaction between the impurity and loratadine (Figure 10).

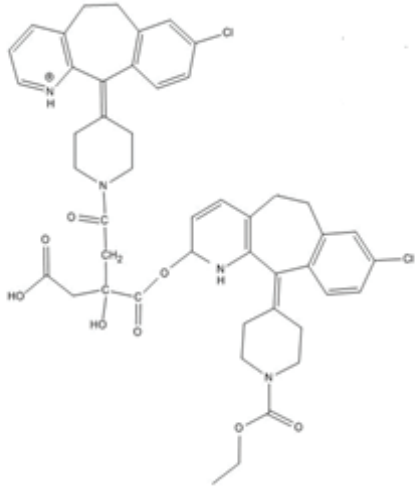


**Figure 10.** Suggested structure of covalent bond between the negative oxygen of the known impurity and the electrophilic carbon connected to the nitrogen of the loratadine molecule.

We might observe that either proposed impurity ion would give us the same experimental results, but the charge-transfer ion is often proposed for colored species in pharmaceutical analysis [24].

## CONCLUSIONS

The results obtained demonstrated that by associating different analytical techniques was possible to control the quality of loratadine raw material, and mass spectrometry using ESI positive mode has become an important analytical methodology in the quality control laboratory for structural identification of pharmaceutical samples.

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