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## Allium cepa.L As Acid-Base Indicator

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In this study, a facile and environmentally friendly method was reported for manufacturing of natural acid-base indicator by preparing *Allium cepa. L* juice, which provided the anthocyanins pigment. The anthocyanins pigment was extracted via boiling process. In detailed, the *Allium cepa. L* was cut into small fragments. Then, the small fragments of *Allium cepa. L* was boiled in distilled water in order to extract the anthocyanin pigment. This process was followed by the addition of different solutions, acidic solution, base solution as well as neutral solution were added into separate test tubes filled with extraction of *Allium cepa. L* juices. The obtained *Allium cepa. L* juice was then used as the pigment for the acid-base indicator. The pH of the solution can be determined by observing the colour change in the *Allium cepa. L* juice. The light purplish colour of *Allium cepa. L* juice turned into red colour when added with hydrochloric acid; its purplish colour of the juice turned into yellow when added with sodium hydroxide; the original colour of *Allium cepa. L* did not undergo any observable colour change when distilled water is added into it. The *Allium cepa. L* exhibited excellent colour change property with chemical solutions. These colour changes make the *Allium cepa. L* be attractive for applications in acid-base indicators.

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## Optimization and validation of non-invasive HPLC-MS/MS method for free-living ruminants stress quantification

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Wildlife management and conservation can benefit from a quantified understanding of physiological response of free-ranging animals to the various potential stressors. Non-invasive stress monitoring by fecal cortisol metabolites determination has proved to be a powerful tool. That is, high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) has emerged as the most accurate method avoiding problems related to the inespecificity and matrix effects of the so-used immunoassays. In this work we have optimized, developed and validated a reliable method for 11-ketoetiocholanolone (11-k), a cortisol metabolite, quantification in ruminant's fecal samples by using and HPLC-MS/MS method. An appropriate extraction and purification procedure was developed to take into account the complex nature of feces. The method consisted in a primary fecal samples extraction with methanol and subsequently clean-up with hexane, followed by purification and preconcentration of targeted metabolite with solid phase extraction (SPE). The final extract obtained was then analyzed by HPLC-MS/MS making use of a quadrupole-time-of-fly (Q-TOF) tandem mass spectrometer with an electrospray ionization interface operating in positive mode. An isotope internal standard was used in order to minimize matrix effect and to compensate the alterations of the analytical signal. After a rigorous optimization of both sample extraction and HPLC-QTOF parameters, the method was satisfactory validated and the best conditions were established. Matrix-matches standards were used for the calibration of the method. The limit of detection and quantification, referred to freeze-dried sample, were 13 and 40 µg kg<sup>-1</sup>, respectively. Recoveries in the range of 85-110% and RSDs not higher than 15% for the complete analytical procedure, including extraction and analysis, were achieved. For the best of our knowledge, this is the first time that the hybrid Q-TOF mass detection coupled to HPLC has been employed for the fecal 11-k quantification in ruminants. Due to the high specificity and sensitivity achieved, the method developed could be used as standard technique for unequivocal fecal 11-k quantification not only in ruminants but also in other related species. In addition, the evaluation of inter-individual differences of stress response could be carried out in the future in an accurate manner by using the present method, thus reducing the effects of potential confounding factors.

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